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EFFECTS OF LOW INTENSITY MICROWAVE RADIATION ON MAMMALIAN SERUM--ETC(U)
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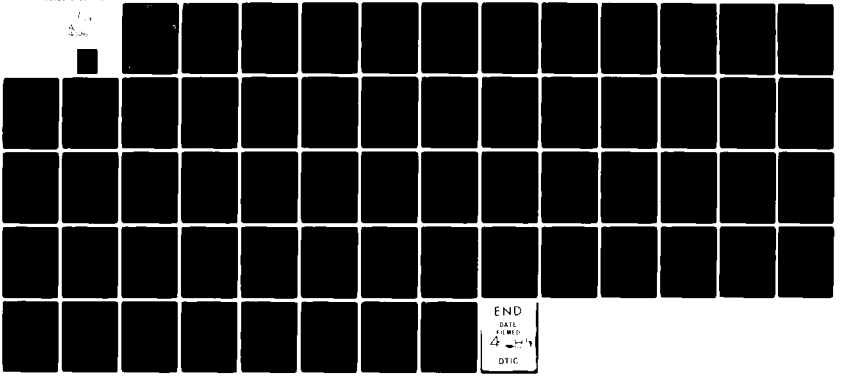

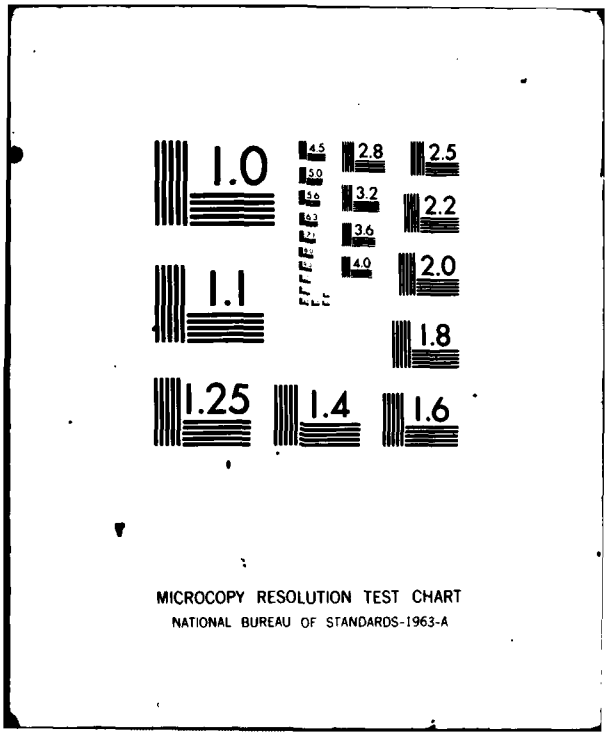
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FINAL REPORT

EFFECTS OF LOW INTENSITY MICROWAVE RADIATION ON MAMMALIAN SERUM COMPONENTS

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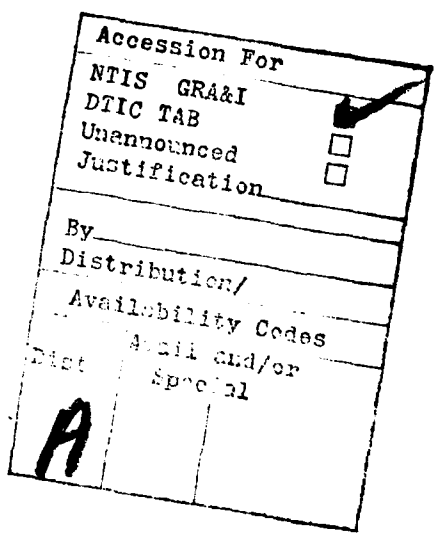
FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.



SUMMARY

In vivo exposure of Dutch rabbits to 1.7 and 2.45 GHz microwave radiation at intensities of 5, 10 and 25 mW/cm^2 resulted in statistically significant dose-dependent alterations in serum glucose, blood urea nitrogen, and uric acid. Other serum components were also found to be altered by such exposure, but not to the extent of these dependent variables. Statistically significant dose-dependent decreases in the duration of sodium pentobarbital-induced sleeping time in the Dutch rabbit were induced by exposure to 1.7 and 2.45 GHz microwave fields in the intensity range of from 5 to 50 mW/cm^2. The 2.45 GHz irradiations produced a statistically significantly greater reduction in sleeping time than exposure at 1.7 GHz at the microwave intensities employed in this investigation. Significant increases in total creatine phosphokinase (CPK) enzyme levels and in CPK isoenzymes were detected in the serum of Dutch rabbits exposed to 2.45 GHz CW microwave fields for two hours at intensities of 10 and 25 mW/cm^2. Serum triglyceride levels were also elevated as a result of such exposure, but the increases were not statistically significant as compared to sham-irradiated controls. The effects of nonradiation heat stress on all of the above dependent variables were investigated by exposing Dutch rabbits to elevated environmental temperatures (35-39°C) for the same duration as the microwave exposures. Qualitative and quantitative differences in the effects of microwave versus nonradiation heat stress were detected in all instances.

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I. INTRODUCTION

Research conducted during the contract year July 1, 1975 to June 30, 1976 has involved a continuation and extension of work which was initiated in July 1972. The overall objectives of this investigation are to develop quantitative assay methods to detect effects of low intensity (ie 5 to 25 mW/cm^2) microwave exposure on mammalian systems. The approach taken has been to develop methods that can be used to screen a large number of physiological response variables to identify those which are most significantly and reproducibly affected by microwave exposure. Once having identified such response variables, they will be used to investigate the basic mechanisms of interaction of microwave fields with biological systems. The identification of microwave exposure sensitive physiological variables that can be measured by in vivo assay techniques also offers a potential means of evaluating the effects of accidental or occupational exposure of humans to microwave radiation. If it is possible to define a set of variables that are consistently altered by such exposure and that are specific responders to microwave insult, a semi-quantitative in vivo method of microwave dosimetry may be developed for application to human exposure hazard evaluations. Background data on such responses, which has appeared in the literature, has emanated from the Soviet Union and other Eastern European nations. This data suggests that specific hematopoietic and biochemical alterations are induced by exposure of experimental animals and humans to microwave fields. The validity of this research has not been ascertained and we have attempted to obtain data that will be pertinent to the evaluation of such reports.

The investigations performed under the terms of this contract have considered the effects of 2.45 and 1.7 GHz continuous wave and pulse modulated microwave fields at intensities of from 5 to 50 mW/cm^2. The categories of effects investigated include biochemical alterations of serum, serum protein changes, analeptic effects, and alterations in serum lipids, triglycerides and enzymes. The general conclusion derived from these in vivo studies of microwave effects on the Dutch rabbit is that microwave intensities in the range employed in this study affect changes in some of the response variables. It has also been determined that, in general, exposure of rabbits to microwave intensities of 10 mW/cm^2 or greater results in detectable manifestations of thermal stress, thus indicating that to a certain extent, at least, the microwave effects involve non-specific stress due to whole body heating. The extent to which the microwave-induced alterations are attributable to thermal stress as contrasted to microwave-specific stress has been the subject of study during the past two years under the terms of this contract. The effects of microwave exposure have been compared with effects of thermal stress administered by exposing experimental animals to elevated environmental temperatures that result in the same increase in deep-colonic temperatures, for the same exposure duration, as in the case of microwave exposure.

This report will consist of a summary of the work performed during this contract period and a review of previous results as pertinent to the discussion of the most recently obtained results. The categories of data to be presented are:

1) comparison of the effects 1.7 and 2.45 GHz irradiations on blood chemistry of rabbits exposed to 5,10, and 25 mW/cm^2 fields for 2 hrs.

2) effects of acute microwave exposures on serum triglycerides and creatine phosphokinase iso-enzymes.

3) results of exposure to these same fields on drug-induced sleeping times in the Dutch rabbit.

II EFFECTS OF 1.7 AND 2.45 GHz MICROWAVE EXPOSURE ON BLOOD CHEMISTRY

PROCEDURE:

The experimental methods employed in the investigation of the effects of 1.7 and 2.45 GHz microwave irradiation have been previously described in detail (see Annual Reports No. 2 and 3 for this contract). Briefly, the procedure consists of serial sampling of the blood of Dutch rabbits by withdrawal of a 4-5 ml sample from the rabbit's marginal ear vein. Samples were obtained from irradiated and sham-irradiated control animals immediately before and immediately post-exposure to the microwave fields for a period of 2 hr. at intensities of 5,10, or 25 mW/cm^2. Blood chemistry analyses were performed by the use of an SMA 12/60 autoanalyzer on the following serum components: calcium, inorganic phosphate, glucose, blood urea nitrogen (BUN), uric acid, cholesterol, total protein, alkaline phosphatase, lactic dehydrogenase (LDH), serum glutamic oxalacetic transaminase (SGOT), total bilirubin, and albumin. The precision of the autoanalyzer was routinely checked on every eighth sample by the introduction of a calibration standard. The precision was also checked by the use of replicate samples from several rabbits which were analyzed periodically on consecutive days to verify the repeatibility of results from identical samples and to ascertain that short-term storage of serum samples did not affect the blood chemistry determinations. The results for albumin are in question since the autoanalyzer assay is a specific dye-binding test for human serum albumin. Apparent differences in dye binding between human and rabbit serum albumin exist such that a quantitative determination of rabbit serum albumin by this method is in doubt. Due to electrical circuit problems in the autoanalyzer during the period in which the effects of 2.45 GHz exposures were being investigated, inaccurate and erratic values of total bilirubin were encountered and thus they are not included in this report.

Groups of 6 or more animals were exposed to the microwave fields at a given frequency and intensity and blood samples were taken at various times post-exposure for periods of up to 7 and 14 days post-exposure to 2.45 and 1.7 GHz fields respectively. In order to minimize the effects of blood sampling stress animals were sampled on alternate days such that samples from at least 3 animals were obtained in all but a few cases where smaller sample sizes were necessitated by the loss of animals from the study. Comparable numbers of sham-irradiated control animals were sampled according to the same schedule as the microwave exposed rabbits.

The microwave irradiations were performed in the far-field in an anechoic chamber at the Department of Microwave Research, Forest Glenn Annex of the Walter Reed Army Institute of Research, Silver Spring, Maryland. Field-intensity and field-distribution calibrations were provided by the staff of the Department of Microwave Research as was the use of laboratory facilities for sample collection and preparation. The contributions to this investigation by the staff of the Department of Microwave Research is gratefully acknowledged.

The effects of microwave exposure on the blood chemistry variables were analyzed by a comparison of the mean values of the exposed ($\bar{X}^T_{i,j}$) and control means $\bar{X}^C_{i,j}$) for the i-th dependent variable on the j-th day of the experiment. The results of the blood chemistry analyses for the irradiated and control animals are shown in Figures 1 to 23, which are plots of the differences between the irradiated and control means (ie $\Delta_{i,j} = \bar{X}^T_{i,j} - \bar{X}^C_{i,j}$) as a function of time pre-and post-exposure to the indicated continuous wave microwave intensities. The values of $\Delta_{i,j}$ for 2.45 and 1.7 GHz fields are plotted in the same figures for sake of comparison and the standard error of the differences is indicated by the error bars.

Studies of the effects of 2.45 GHz microwave fields, which were conducted during the initial phase of the investigation, involved the immobilization of the rabbit during exposure by lightly taping the animal to a board and taping the rear and hind legs together to restrict motion during the 2 hr. exposure period. The animals were thus placed upright in the prone position with their long axis (ie. length) perpendicular to the microwave beam axis which intersected the animal at its midline. In these and subsequent exposures to 1.7 GHz fields the electric field was vertically polarized. The temperature of the exposure chamber was thermostatically controlled at 70 $\pm$ 2°F during exposures and the relative humidity varied from 40 to 60%.

In an attempt to more closely simulate the conditions of human exposure and to reduce the stress due to constraint, the animals exposed to 1.7 GHz microwaves were not constrained during microwave exposure. During exposures at this frequency the rabbits were placed in a styrofoam box consisting of a base 24" by 18" with four 2" square 12" vertical sytrofoam posts at each corner. Monofilament nylon line was wound around the periphery of the box to keep the animal in the enclosure during irradiation. In this case, therefore, the rabbit was free to move about the confines of the box during exposure and there was free circulation of air thus minimizing any thermal effects of constraint. The variation in the mode of constraint of the animals exposed to the two frequencies (1.7 and 2.45 GHz) must be taken into account in comparing the responses of the animals to the microwave frequencies used in this investigation. At each frequency, sham-irradiated control animals were subjected to the identical mode of constraint as the irradiated rabbits thus controlling for the differences in constraint-induced stress. A comparison of constraint-induced stress effects was made by exposing animals to the same type of constraint during irradiation at 2.45 and 1.7 GHz and determining the effects of microwave-induced thermal stress by recording the temporal course of rectal temperature elevations. No statistically significant difference was detected between the rectal temperature response of animals irradiated at a given frequency and subjected to the two types of constraint, but the trend in the response suggested that immobilization tended to decrease the magnitude of the mean rectal temperature during irradiations at 10 mW/cm^2. This finding is attributed to the fact that the immobilized animals did not move about in the field to minimize the microwave-induced thermal stress as contrasted with the unrestrained animals who exhibited an increased avoidance reaction which resulted in a slightly higher rectal temperature than in immobilized animals exposed to the same intensity and frequency of microwave radiation. The activities of rabbits exposed to microwave radiation, at either frequency, was observed via closed circuit television. At intensities of 10 mW/cm^2 the irradiated animals were generally more active during exposure than the sham-irradiated controls. This was interpreted as an avoidance

reaction elicited by thermal stress which at this intensity resulted in a mean rectal temperature elevation of approximately I°C at the end of two hours of exposure. The behavior of animals exposed to fields of 25 mW/cm^2 differed from that at 10 mW/cm^2 in that at the higher power density the animals displayed very active avoidance reactions during the first hour of exposure, during which time the mean rectal temperature elevation was approximately 1.7°C. During the second hour of exposure the animals generally became significantly less active and at the end of two hours of exposure they assumed a prone position and showed signs of heat stress as expected on the basis of the 2.5°C rectal temperature rise that was elicited by such exposure. There were no obvious differences in the activity of animals exposed at 5 mW/cm^2 relative to sham-irradiated control rabbits. The difference in activity of animals exposed to 10 or 25 mW/cm^2 1.7 GHz microwave fields relative to exposures at 2.45 GHz of constrained animals, must be considered as an indication of a constraint-irradiation interaction. Although the use of sham-irradiation would provide internal control for a given type of animal constraint, the constraint-irradiation interaction must be considered in a cross-comparison of the effects of 1.7 and 2.45 GHz microwaves as employed in this investigation.

RESULTS AND ANALYSIS

The statistical significance of the difference in the mean responses of irradiated and control animals is determined by means of Student's t test. The test statistic is defined as

$$t = \Delta_{i,j} \{ s_p \left(\frac{1}{n_T} + \frac{1}{n_C}\right)^{\frac{1}{2}} \}^{-1}$$

and

$$\Delta_{i,j} = \bar{X}^T_{i,j} - \bar{X}^C_{i,j}$$

n_T = number of irradiated animals

n_C = number of control animals

s_p = pooled estimate of the standard deviation of $\Delta_{i,j}$

$$= \{(n_T - 1)\, s_T^2 + (n_C - 1)\, s_C^2 / (n_T + n_C - 2)\}^{\frac{1}{2}}$$

where s_T^2, s_C^2 are the estimated sample variances for the microwave exposed and sham-irradiated controls respectively. The null hypothesis (ie. $H_o: \Delta_{i,j} = 0$) is rejected at the 5% level of significance (ie $p < 0.05$). The values of $\Delta_{i,j}$ that are statistically significant at the 5% level are indicated by a single asterisk (*) on the graphs of $\Delta_{i,j}$ versus time; values significant at the 1% (or less) level are indicated by a double asterisk (**). In the cases where the dependent variable exhibited an apparent microwave dose-dependency a dose-response curve was drawn by an eye-fit of the data points. Since this method was used only as an aid in the interpretation of the data no attempt was made to determine the statistical significance of the trends exhibited by the various dose-response relationships.

In presenting the temporal behavior of the various dependent variables, the differences in the mean values are plotted as a function of time, with the abscissal values corresponding to the days on which the serum samples were drawn. As previously noted, the sampling intervals were not the same in all cases due to limitations on the frequency of sampling from a given animal and the total sampling interval was limited to 7 days in the 2.45 GHz exposures

and 15 days following exposure at 1.7 GHz. In order to facilitate comparison of response patterns to the two microwave frequencies, the means for a given variable have been connected by line segments. The transient response of some of the variables to microwave exposure is such that in cases where the variables were not measured on the same days, a comparison of the response patterns for data separated by intervals of one or more days may obscure similarities in responses in this type of comparison. As a consequence of this fact, the response pattern comparisons are generally most useful only for the interval of most frequent sampling for both frequencies (ie. up to 3 days post-exposure). The data obtained in this study which showed a microwave-dependent response is included in Figures 1 to 23. The dependent variables which indicated statistically significant responses to microwave exposure will be discussed in this report.

GLUCOSE

The differencesin serum glucose levels between rabbits exposed to 25,10, and 5 mW/cm^2 microwave radiation at frequencies of 1.7 and 2.45 GHzare shown in Figures 1, 2, and 3 as a function of time pre-and up to 15 days post-exposure. This dependent variable exhibited the most significant and consistent response to microwave exposure at either frequency. The response pattern is characterized by a maximum elevation in the immediate post-exposure samples at all intensities investigated, followed by a decrease to normal or below normal values on day 2, followed by another increase by day 3. Following these initial oscillations the 2.45 GHz levels were significantly decreased 7 days post-exposure, whereas the 1.7 GHz irradiation resulted in normal values at day 6 except at 25 mW/cm^2 in which case a statistically significant increase was detected. The initial increase in glucose levels immediately post-exposure is most likely an indication of a physiologic response to acute stress due to the mobilization of liver glycogen reserves as mediated by epinephrine release as a sympathetic nervous system response. The biphasic response of serum glucose levels to microwave radiation may be an indication of transient renal proximal tubule injury leading to a deficiency in glucose reabsorption in the tubules. The immediate post-exposure depression in serum glucose following exposure to 1.7 GHz at 10 mW/cm^2 is attributed to an abnormally high value in a sham-irradiated control and the large standard deviation of the difference in irradiated and control means reflects this effect. Excluding this non-statistically significant response, a dose-response curve relating the difference in glucose levels to microwave power density immediately post-exposure is obtained as shown in Figure 4. Although the 2.45 GHz exposures appeared to produce a greater elevation than the 1.7 GHz microwave field, the difference in the dose-response curves corresponding to these frequencies is not statistically significant. Although the significance of a comparison of the 1.7 GHz data at day 6 with the 2.45 GHz data on day 7 is questionable due to the transient nature of the response, it may be suggested that the recovery of animals exposed to 25 mW/cm^2 is not in phase for the two frequencies investigated, perhaps as a result of the differences in modes of constraint previously mentioned. By the 11th day post-exposure serum glucose levels returned to normal following 1.7 GHz microwave exposure.

BLOOD UREA NITROGEN

The mean differences in blood urea nitrogen (BUN) for rabbits exposed to 25, 10 and 5 mW/cm^2 at 1.7 and 2.45 GHz microwaves and sham-irradiated

controls are summarized in Figures 5,6, and 7 respectively. The response patterns following 2 hr exposures to 25 and 10 mW/cm^2 at a frequency of 2.45 GHz are similar in that biphasic alterations occur in both cases with an increase in BUN immediately after exposure, followed by highly statistically significant decreases in BUN relative to controls on day 1 post-exposure, followed by a second increase by day 2 and eventually resulting in decreased BUN levels 7 days post-exposure. At an intensity of 5 mW/cm^2 the 2.45 GHz exposure BUN levels followed the same trend for the immediate post-exposure and 1 day samples, but on day 2 a significant decrease in BUN was noted, with a return to normal values by day 7. With the exception of this unexplained response at 5 mW/cm^2 on day 2, the BUN response patterns are similar in nature to the serum glucose patterns in that both dependent variables exhibited biphasic responses to microwave irradiation with the same general phase relationship resulting in small but statistically significant decreases in BUN 7 days post-exposure. The BUN response to 1.7 GHz exposure at 25 mW/cm^2 is again seen to follow the same general biphasic pattern, although in this case the alterations are not statistically significant due, most likely, to the small sample size and consequent large standard deviations. Exposure to 1.7 GHz microwaves at intensities of 10 and 5 mW/cm^2, as shown in Figures 6 and 7, produces similar patterns of non-statistically significant variations in BUN but no decrease in BUN is seen to result on day 1 post-exposure. The dose-response curves plotted in Figure 8 for the immediate-post exposure data indicate general agreement between the effects of 1.7 and 2.45 GHz radiation on BUN levels at this sampling time, again suggesting that the acute stress response, as reflected by this variable, is similar at these frequencies. At either frequency it appears that the initial post-exposure response is not significantly affected by radiation intensities of 5 or 10 mW/cm^2 but increases significantly and to approximately the same levels at either exposure frequency following a 2 hr. exposure at 25 mW/cm^2. The immediate post-exposure elevation of BUN is characteristic of the alarm-reaction phase of response to acute stress. BUN elevations may result from protein catabolism or decreased rates of protein synthesis, the latter being more commonly associated with thermal stress. Hyperthermia also decreases renal urea clearance and dehydration is known to result in increased BUN, thus the mechanism(s) responsible for microwave-induced increases in BUN are uncertain at this time. The explanation of the significant decrease in BUN 24 hrs. post-exposure is also indefinite since this could result from a number of phenomena including rebound protein synthesis, hepatic insufficiency, or excess imbibition of fluids in response to microwave induced dehydration.

URIC ACID

Serum uric acid levels were elevated in the immediate post-exposure samples in animals exposed to 1.7 and 2.45 GHz microwaves at intensities of 25 and 10 mW/cm^2 as shown in Figures 9 and 10 respectively. The transient elevation is followed by a decrease in uric acid levels in one and two day post-exposure samples. Irradiations with 2.45 GHz radiation at 25 mW/cm^2 resulted in a statistically significant elevation 7 days post-exposure suggesting a biphasic response as previously detected in glucose and BUN levels. Irradiation at 5 mW/cm^2 at a frequency of 2.45 GHz produced a depression of uric acid levels to below control values which persisted for the 7 day sampling interval. The variation in the dose-response relationship at 1.7 and 2.45 GHz are shown in Figure 12. Five milliwatt per square centimeter exposure at both frequencies resulted in a non-statistically significant decrease in serum uric acid in immediate post-exposure samples. Irradiation at 10 mW/cm^2 resulted in elevations

in uric acid, whereas at 25 mW/cm^2 the 2.45 GHz field led to a further increase in uric levels. Irradiation with 1.7 GHz microwaves at 25 mW/cm^2 resulted in a decrease in uric acid relative to the 10 mW/cm^2 level which was not, however, statistically significantly different from the response at 10 mW/cm^2. The dose-response relationships suggest that low intensity exposure results in a qualitatively different response than exposures at 10 or 25 mW/cm^2. In humans, serum uric acid levels are related to purine catabolism which is not the case in rabbits, thus suggesting that these results are most likely related to altered excretion due to renal involvement or to microwave-induced dehydration. The uric acid response in animals irradiated at 5 mW/cm^2, which did not produce evidence of thermal stress as indicated by rectal temperatures, would tend to support the hypothesis that the immediate post-exposure transient increase in serum uric acid was due to the effect of microwave heat stress which was detected as a consequence of exposures to either 1.7 or 2.45 GHz microwaves at intensities of 10 or 25 mW/cm^2.

CHOLESTEROL

The effects of 1.7 and 2.45 GHz CW microwaves on serum cholesterol are shown in Figures 13-16. The immediate post-exposure values are increased at either radiation frequency with a definite dose-response relationship exhibited in the 2.45 GHz data at which frequency larger elevations in serum cholesterol are induced than at 1.7 GHz. Transient depression in serum cholesterol occurred at 1 day post exposure following exposures to 2.45 GHz at 5, 10, and 25 mW/cm^2 and at 5 and 25 mW/cm^2 at a frequency of 1.7 GHz. Elevations of cholesterol may be attributable to dehydration or altered kidney function due to a decrease in the serum lipoprotein lipase activity, or excessive hepatic synthesis of cholesterol esters to compensate for low albumin. Decreased cholesterol values which occurred one or more days post exposure, suggest possible liver involvement or a neuroendocrine response involving the stimulation of thyroid activity.

SERUM GLUTAMIC OXALACETIC TRANSAMINASE (SGOT)

Serum glutamic oxalacetic transaminase (SGOT) is known to be elevated by alterations in cell membrane permeability or cellular destruction associated with various types of physiological stress including prolonged and severe exercise. Acute stress results in increased levels in human serum which reach maximum values within 48 hours and return to normal within 3 to 5 days. The effects of microwave irradiation at frequencies of 1.7 or 2.45 GHz are shown in Figures 17-20. A general increase in SGOT levels immediately post-exposure was induced by exposure at both microwave frequencies with a return to normal values by 3 to 7 days post-exposure. SGOT microwave-dose response for the 1.7 GHz field is shown in Figure 20. A dose-and time-dependent increase is noted during the first 24 hrs. post exposure period which is consistent with an acute stress response. The response patterns to the two microwave radiation frequencies exhibit the same general trends.

LACTIC DEHYDROGENASE (LDH)

The effects of a single 2 hr. exposure to 1.7 and 2.45 GHz microwave radiation at intensities of 25 or 10 mW/cm^2 on alterations in LDH levels are shown in Figures 21 and 22. The data obtained from animals exposed to 1.7 GHz was highly variable such that no statistically significant differences

were detected with the exception of a significant elevation one day post-exposure to an intensity of 10 mW/cm^2. Statistically significant increases in LDH following exposure at 2.45 GHz occurred immediately post-exposure and one day post-exposure to 25 mW/cm^2; on day two post-exposure there was a significant decrease in LDH in irradiated animals which also occurred at one day post-exposure to a 10 mW/cm^2 field. There were no statistically significant alterations due to exposure to either frequency at an intensity of 5 mW/cm^2. LDH is an intracellular enzyme and elevations in the serum are generally due to cell death, altered cell membrane permeability, and since it functions in the glycolytic cycle to catalyze the conversion of lactic acid to pyruvic acid, LDH levels are increased following physical stress involving skeletal muscle activity. The increase in LDH induced by microwave exposure is attributed to a general stress-induced avoidance response. The increased levels may be related to tissue-specific microwave damage since LDH exists as five isoenzymes with varying concentrations in different tissues such as the heart, kidney, lung, and liver.

TOTAL BILIRUBIN

The variation of the difference in total serum bilirubin levels in rabbits exposed to 1.7 GHz CW microwave radiation at an intensity of 25 mW/cm^2 and sham-irradiated controls is shown in Figure 23. As previously mentioned, equipment malfunction during the analysis of the 2.45 GHz data precluded the determination of bilirubin levels. Figure 23 indicates statistically significant increases in bilirubin in the immediate post-exposure and one day post-exposure samples followed by a return to normal values by day 3. Increased total serum bilirubin is an indication of dehydration, liver or kidney damage, or hemolysis. Elevated bilirubin causes an artifactural lowering of albumin when albumin is determined by the Haba Dye Method which is used in the SMA 12/60 Auto-analyzer,which suggests the possibility that the bilirubin elevation may have supressed microwave-induced alterations in serum albumin. Elevated serum bilirubin also produces artifactural elevation of cholesterol which may have affected the previously described microwave response of serum cholesterol levels.

The remaining dependent variables, namely: inorganic phosphate, alkaline phosphatase, total protein, albumin, and calcium either did not exhibit discernable microwave irradiation effects or were subject to experimental difficulties that rendered data analyses difficult or impossible. The normal serum calcium levels in Dutch rabbits is on the order of 15 mg% compared to a mean level of 9.5 mg% in humans. The SMA 12/60 autoanalyzer range for serum calcium determinations is 0 to 15 mg%, thus the rabbit levels lie at the upper limit of analysis and the data is of questionable usefulness.

The results of the blood chemistry determinations indicate that microwave exposure at either 1.7 or 2.45 GHz leads to dose related alterations in a number of dependent variables, most significantly glucose, BUN, and uric acid. Based upon the finding that microwave exposure at either frequency and at intensities of 10 mW/cm^2, or greater, results in statistically significant rectal temperature elevations (the time course of which is consistent with the alarm-reaction-resistance-exhaustion response) it could be concluded that alterations of serum components are related to the effects of thermal stress. Since data is not available on the effects of thermal stress on the rabbit

serum components analyzed in this study, it was necessary to study the effects of generalized thermal stress on the Dutch rabbit's serum chemistry. Serum chemistry alterations were consequently investigated by exposing four Dutch rabbits to an elevated environmental temperature of 39°C for two hours in a temperature-controlled environmental chamber. This exposure produced a mean rectal temperature elevation of 2.1 ± 0.37 °C, which is approximately the same mean elevation induced by a two hour microwave exposure at 25 mW/cm^2. A control group of four Dutch rabbits was exposed for 2 hrs. at room temperature (22°C) in the same chamber with a mean rectal temperature elevation of 0.1 ± 0.15 °C. Immediately following heat or sham exposure, serum samples were obtained and analyzed on the SMA 12/60. The means and standard errors of the means are shown in Table 1 along with the Student's t value and level of significance for those variables that were significantly affected by heat exposure. Statistically significant decreases in serum calcium, inorganic phosphate and albumin were induced by exposure of the Dutch rabbits to environmental heat stress. Although not statistically significant, levels of the serum enzymes alkaline phosphatase, lactic dehydrogenase, and serum glutamic oxalacetic transaminase were all elevated. On the basis of these results it may be concluded that the effects of microwave-induced thermal stress are not the same in the Dutch rabbit as heat stress that results in the same approximate rectal temperature elevation during a two hour exposure to either stress.

In an attempt to gain some additional insight into the nature of the effects of microwave exposure on the Dutch rabbit, as manifested by alterations in serum components, a qualitative comparison of the profiles of the SMA 12/60 changes corresponding to microwave exposure, dehydration, liver damage (cirrhosis), kidney damage (nephrotic syndrome), heart damage (myocardial infarction) and heat stress at 39°C is presented in Table 2. The alterations for each dependent variable for each type of physiological stress are qualitatively scored to indicate similarities in response. The utility of this comparison is obviously somewhat limited by the fact that the scoring for microwave and heat exposure pertains to the Dutch rabbit results obtained in this study, whereas the other categories of physiological alterations are obtained from human disease entities.

This comparison of response profiles suggests that the acute response to microwave exposure (10 mW/cm^2 or greater) as employed in this study somewhat resembles dehydration or kidney damage with some common factors with liver damage as well. The results of histopathological examinations of rabbits exposed to 2.45 GHz microwaves for 2 hours at 25 mW/cm^2 indicated that such exposure resulted in acute renal tubular nephrosis with maximal damage to epithelial cells of the proximal convoluted tubules. This effect could be due either to direct microwave insult from localized heating or dehydration nephrosis. The possibility that 2.45 GHz microwaves and to a somewhat lesser extent 1.7 GHz microwaves, interact with the Dutch rabbit in a different manner than thermal stress is suggested on the basis of a comparison of the response profiles of microwave and heat stressed rabbits. The possibility that microwave radiation induces tissue-specific damage which is qualitatively and quantitatively different from environmental heat stress was further investigated by the analysis of creatine phosphokinase isoenzymes.

III. CREATINE PHOSPHOKINASE ISOENZYME ALTERATIONS BY MICROWAVE AND HEAT STRESS

Comparison of the response profiles of serum components in the rabbit

TABLE 1. EFFECT OF ENVIRONMENTAL HEATING ON DUTCH RABBIT SERUM CHEMISTRY

	Ca^{++} mg%	I.P.* mg%	Glucose mg%	BUN mg%	Uric Acid mg%	Chol.*** mg%	T.P.** gm%	Albumin gm%	Bilir. mg%	Alk. Phosp. mU/ml	LDH mU/ml	SGOT mU/ml	
CONTROLS (Room temp) 22°C, 2 hr $\bar{X}^C$	14.4	4.3	157.3	18.8	0.8	33.8	6.4	4.6	0.2	39.3	46.3	18.8	
St. Error of $\bar{X}^C$	0.2	0.1	5.1	2.5	0.04	3.8	0.2	0.1	0.1	7.8	9.0	2.3	
HEATED 39°C, 2 hr $\bar{X}^T$	13.7	3.8	152.8	15.8	0.7	31.5	6.2	4.3	0.2	49	56.5	27.2	
St. Error of $\bar{X}^T$	0.1	0.3	6.8	1.6	0.1	3.1	0.2	.03	0.04	6.1	19.3	6.1	
$\Delta = \bar{X}^T - \bar{X}^C$	-0.6	-0.6	- 4.5	-3	-.12	-2.3	-0.2	-0.3	0	9.75	10.25	8.45	
t value	2.8	2.1	--	1.0	1.0	--	--	2.2	--	1.0	0.5	1.3	
level of significance (p)	0.012	0.04	--	0.18	0.18	--	--	0.04	--	0.18	0.32	0.121	

* I.P. = inorganic phosphate
** T.P. = total protein
***Chol. = cholesterol

TABLE 2

SERUM CHEMISTRY PROFILES IN RESPONSE TO MICROWAVE RADIATION AND PHYSIOLOGICAL ALTERATIONS

Dependent Variable	Microwave Exposure* Acute Response	Dehydration**	Liver Damage** (Cirrhosis)	Kidney Damage** (Nephrotic Syndrome)	Heart Damage ** (Myocardial Infarction)	Heat Stress* 39°C 2 hrs.
Serum Albumin	0	++	--	--	0	-
BUN	++	++	--	++	++	-
Total Protein	0	++	-	--	0	-
Ca^{++}	0	++	--	--	0	-
Uric Acid	++	++	0	++	++	-
Glucose	++	++	++	++	++	-
Inorganic Phosphate	+	+	--	++	0	-
Cholesterol	++	++	-	++	++	-
Total Bilirubin	+	+	++	+	+	+
Alkaline Phosphatase	0	+	++	++	0	+
LDH	+	+	+	++	+++	+
SGOT	++	+	++	-	+++	+

0 = no change
\+ = slight increase
++ = significant increase
\- = slight decrease
-- = significant decrease

* indicates Dutch rabbit response
** indicates human response

following microwave or heat stress suggested the possibility that microwaves produce differential tissue-specific alterations. To obtain additional data to support this hypothesis the effects of microwave and heat stress on creatine phosphokinase isoenzymes (CPK) in rabbits were investigated. The procedure was similar to that used in studies of other serum components with the exception that, to date, only acute responses have been investigated. Groups of rabbits were exposed to 2.45 GHz microwaves at intensities of either 10 or 25 mW/cm^2 for two hours after which time a serum sample was immediately obtained for analysis. Rectal temperature alterations were determined for all irradiated and sham-irradiated controls. In another series of experiments, rabbits were placed in a temperature controlled environmental chamber for 2 hrs. at ambient temperatures of 35 to 39°C to induce rectal temperature elevations of the same order of magnitude as microwave irradiation. Heat exposure was again followed by serum sampling.

The CPK isoenzymes, which are denoted a MM,MB, and BB, are found in differing concentrations in body tissues. The MM isoenzyme is found primarily in skeletal muscle; MB primarily in heart muscle and the BB fraction is associated with brain tissue. Analysis of alterations in CPK isoenzymes in serum thus offers a potential means of detecting tissue-specific microwave effects. In this investigation the method of analysis of CPK isoenzyme follows that described by Nealon and Henderson.[1] The results of the CPK isoenzyme studies on microwave-irradiated and heat-stressed animals are summarized in Table 3 and the results of a statistical analysis (Student's t test) of the differences in group treatment means is summarized in Table 4. CPK isoenzyme levels are affected by storage time between sampling and analysis as well as the metabolic status of the animal. Since various storage times were used in different experiments and experiments involving animals that had been fasted for 24 hr. prior to treatment and unfasted animals were involved in these studies, the treatment groups must be compared with the appropriate control groups as indicated in Table 4.

It may be concluded that CPK isoenzyme levels are elevated in a dose-dependent manner by exposure to 2.45 GHz microwave radiation at intensities of 10 or 25 mW/cm^2 following a 2 hour exposure. Exposure of rabbits to a heated environment (39°C) for the same exposure duration resulted in the same mean rectal temperature elevation as 25 mW/cm^2 microwave exposure and similar although non-statistically significant increases in enzyme levels. Whereas 25 mW/cm^2 exposure resulted in a 114% increase in total CPK isoenzymes, exposure to 39°C for the same duration increased the total CPK isoenzyme level by only 74%. Differences in the effects of these treatments were also reflected in brain-specific CPK, BB fraction which was increased by 163% as a result of microwave exposure compared to a 58% increase due to 39°C heat exposure. Exposure to 10 mW/cm^2 microwave radiation produced a mean rectal temperature elevation of 0.27°C as compared to 0.69°C and 0.71°C elevations following exposure to elevated environmental temperatures of 35°C and 37°C respectively. The 10 mW/cm^2 exposure, however, led to statistically significant elevations in MM,MB, and total CPK isoenzymes which were not significantly elevated by heat stress that resulted in significantly higher rectal temperature elevations. It may thus be concluded that 2.45 GHz microwave exposure at 10 and 25 mW/cm^2 induces qualitatively and quantitatively different alterations in CPK isoenzyme levels than heat exposure suggesting tissue-specific responses to microwave fields.

1. Nealon, D.A. and Henderson, A.R., Clinical Chem. 21, 392 (1975).

TABLE 3. EFFECT OF MICROWAVE IRRADIATION AND HEAT ON RABBIT CPK ISOENZYMES

Experimental Conditions	Mean(±S.E.M.)Rectal Temperature Change (°C)	Mean(±S.E.M.)CPK Isoenzyme Levels (U/l)			
		MM	MB	BB	Total
GROUP A Sham-irradiated 2 hr (fasted 24 hr) n = 6	0.10±0.08	34.6±6.2	11.6±1.5	31.5±5.3	77.7±10.7
GROUP B 2.45 GHz, 10mW/cm^2; 2 hr. exposure (fasted 24 hr) n=3	0.27±0.12	64.2 ± 4.5	18.3±1.0	30.9±5.1	113.4±3.5
GROUP C 2.45 GHz, 25mW/cm^2; 2hr. exposure (fasted 24hr) n=3	2.17±0.13	65.8±10.6	17.4±0	83.0±48.7	166±59.2
GROUP D Room temp. (22°C) in environ. chamber, 2hr. n=4	0.10±0.15	55.7±6.5	13.0±1.5	50.7±22.6	119.4±18.0
GROUP E Heated (39°C) in environ. chamber n = 4	2.1±0.37	109±34.7	18.8±5.5	80.3±49.1	208.1±60.2
GROUP F Room temp. (22°C) environ. chamber 2hr. n=3	0.13±0.20	84.43±12.2	18.4±1.0	102.3±29.3	205.1±23.9
GROUP G Heated (35°C) in environ. chamber, 2hr; n=4	0.69±0.25	116.5±52.1	24.3±8.5	55.0±31.9	195.8±89.0
GROUP H Room temp. (22°C) in environ. chamber, 2 hr; n=4 (fasted 24 hr)	-0.20±0.08	106.4±43.3	29.7±8.7	78.9±38.0	214.9±60.2
GROUP I Heated (37°C) in environ.chamber; 2hr; n=4 (fasted 24 hr)	0.71±0.15	86.1±17.8	24.6±6	88.3±44.4	199±58.9

Table 4 STATISTICAL ANALYSIS OF CPK ISOENZYME ALTERATIONS

(Student's t Test)

GROUP COMPARISONS	Change in Enzyme Levels (Treated - Control)											
	MM			MB			BB			TOTAL		
	Δ	t	p	Δ	t	p	Δ	t	p	Δ	t	p
B vs A	29.6 (86)*	3.1	.009	6.7 (58)	3.0	.01	-0.6 (-2)	0.1	.46	35.7 (46)	2.2	.03
C vs A	31.2 (90)	2.7	.015	5.8 (50)	2.6	.018	51.5 (163)	1.6	.07	88.3 (114)	2.1	.037
E vs D	53.5 (96)	1.5	.09	5.8 (45)	1.0	.18	29.6 (58)	0.6	.29	88.7 (74)	1.4	.11
G vs F	32.1 (38)	0.5	.32	5.9 (32)	0.6	.29	-47.3 (-46)	1.1	.16	-9.3 (-5)	0.1	.47
I vs H	-20.2 (-19)	0.4	.35	-5.1 (-17)	0.5	.32	9.4 (12)	0.2	.42	-16 (-7)	0.2	.42

( )* = % change in enzyme concentration

$$= \frac{\text{(treated) - (control)}}{\text{(control)}} \times 100$$

IV SERUM TRIGLYCERIDES

Further insight into the question of the physiological response of mammalian systems to microwave exposure was sought by determining the effects of exposure on serum triglyceride levels. The purported involvement of the mammalian neuroendocrine system in microwave-induced alterations suggests the possibility that such exposure can result in the release of the peptide growth hormone (GH) from the pituitary gland. Growth hormone is known to antagonize the effects of insulin by inhibition of cellular uptake of glucose thus leading to increased serum glucose as detected in this investigation following microwave exposure to 2.45 and 1.7 GHz fields. This hormone also causes the release of free fatty acids from tissue storage deposits which could consequently cause elevations of serum triglycerides.

Rabbits were exposed to 10 or 25 mW/cm^2 intensities of 2.45 GHz CW microwaves for 2 hrs. Rectal temperatures were determined immediately prior to and after microwave exposure and a serum sample was taken from the marginal ear vein for triglyceride analysis. The triglycerides were extracted from serum using a modified Dole procedure which involves partition of serum lipids between a polar and a non-polar liquid. Triglycerides remain in the nonpolar phase while phospholipids, glucose, glycerol and other polar molecules will be taken up in the polar solvent. Glycerol is liberated from triglycerides by transesterification using sodium ethoxide and is then oxidized with sodium periodate to formaldehyde. Color is then developed with acetylacetone and triglyceride levels are determined by spectrophotometry.

To provide an indication of the effects of nonradiation heat stress, groups of rabbits were exposed for 2 hr to an elevated environmental temperature of 36°C in an environmental chamber and thermal controls were exposed for the same duration and in the same chamber maintained at normal room temperature (22°C). Pre- and post-treatment rectal temperatures were again determined and serum samples obtained for triglyceride analyses.

The results of the triglyceride determinations in microwave-and heat-stressed Dutch rabbits, together with mean rectal temperature elevations, are presented in Table 5. The statistical analyses, utilizing the Student's t test, are summarized in Table 6 together with the percentage change in serum triglyceride levels induced by microwave radiation or heat stress. Although none of the treatment effects were found to be statistically significant at the 5% level, microwave exposure at intensities of 10 or 25 mW/cm^2 for 2 hrs appears to result in a greater elevation of serum triglycerides than heat stress. In the case of 10 mW/cm^2 exposures the mean rectal temperature elevation was significantly less than in heat-stressed animals but the percentage increase in serum triglycerides following microwave exposure is greater than that due to heat stress. The variability of the triglyceride levels and, probably of more importance, the small sample sizes used in this preliminary analysis reduced the statistical significance of these findings but there is a definite indication that 2.45 GHz microwave exposure for 2 hrs at 10 or 25 mW/cm^2 results in a more pronounced elevation of serum triglycerides than heat stress as employed in this investigation.

A more detailed investigation of the effects of heat stress and 10 mW/cm^2 exposure at 2.45 GHz on serum lipids is in progress. Serum samples from heat stressed rabbits (2hrs at 37°C), room temperature controls, sham-irradiated controls (22°C) and rabbits exposed to 2.45 GHz microwaves for 2 hr at an

Table 5. EFFECT OF MICROWAVE EXPOSURE AND HEAT-STRESS ON RABBIT SERUM TRIGLYCERIDES

TREATMENT GROUPS	MEAN AND S.E.M. RECTAL TEMP. CHANGE (^{o}C)	NUMBER OF RABBITS	MEAN AND S.E.M. SERUM TRIGLYCERIDES (mg/dl) (Immediately post-treatment)
Group 1: Sham-irradiation, room temp (22^{o}C); 2hr.; 24 hr fasted	0.01 ± 0.07	6	40.3 ± 8.4
Group 2: 2.45 GHz CW; 10mW/cm^2 2 hr.; 24 hr fasted	0.27 ± 0.12	3	57.6 ± 10.3
Group 3: 2.45 GHz CW; 25mW/cm^2 2hr.; 24 hr fasted	2.17 ± 0.13	3	56.5 ± 6.4
Group 4: room temp. (22^{o}C); environ chamber, hr.; 24 hr. fasted	-0.20 ± .07	4	24.4 ± 13.4
Group 5: Heat stressed; (36^{o}C); environ chamber; 2 hr; 24 hr fasted	0.71 ± 0.16	4	28.8 ± 7.8
Group 6: room temp. (22^{o}C); environ. chamber; 2hr; non-fasted	0.05 ± 0.16	4	46.4 ± 16.1
Group 7: Heat stressed; (36^{o}C); environ.chamber; 2hr.; non-fasted	0.54 ± 0.30	3	25.3 ± 7.5

Table 6. STATISTICAL ANALYSIS OF SERUM TRIGLYCERIDE DATA (STUDENT'S t TEST)

GROUP COMPARISONS	DIFFERENCE IN TRIGLYCERIDES (Treated - Controls) (mg/dl)		t-STATISTIC (d.f.)	p VALUE (level of significance)
Group 2 vs Group 1	17.30	(+43%)*	1.24 (7)	0.13
Group 3 vs. Group1	16.20	(+40%)	1.25 (7)	0.12
Group 5 vs. Group 4	4.40	(+18%)	0.28 (6)	0.39
GROUP 7 vs Group 6	-21.1	(-45%)	1.06 (5)	0.16

*% Change in Serum Triglyceride Levels = $\frac{\text{Treated - Control}}{\text{Control}} \times 100$

intensity of 10 mW/cm^2 have been obtained. These serum samples will be analyzed for triglycerides, lipids , free fatty acids and cholesterol.

V. EFFECTS OF 2.45 AND 1.7 GHz MICROWAVE RADIATION ON PENTOBARBITAL-INDUCED SLEEPING TIME

The effects of 2.45 and 1.7 GHz microwave radiation at intensities of from 5 to 50 mW/cm^2 on sodium pentobarbital-induced sleeping time in the Dutch rabbit have been investigated in an attempt to obtain information on the mechanisms of interaction of low-intensity microwave fields with mammalian systems. The results of this study indicate that there is a statistically significant decrease in the duration of sleeping time as a result of exposure to either frequency of microwave radiation. The mechanism for the microwave-induced reduction in sleeping time was investigated by analyzing the relationship between sleeping time and microwave-induced thermal stress using the rabbit rectal temperature as the dependent variable. The results of this study, which are presented in Appendix A of this report, indicate that the analeptic effect of microwave exposure is due, at least in part, to the thermal stress imposed on the animals.

It was found, however, that exposures to 2.45 GHz fields resulted in statistically significantly greater reductions in sleeping time than exposures to the same intensity field at 1.7 GHz. This finding is not consistent with a direct thermal phenomenon, since the results of Gandhi[2] would suggest that although the total power deposition in a rabbit exposed to 1.7 or 2.45 GHz microwave fields would not differ appreciably at these frequencies, greater absorption should theoretically occur at 1.7 GHz than at 2.45 GHz. In an attempt to resolve this difference in frequency response, further studies have been conducted to obtain additional data relative to the mechanisms involved in the analeptic effect of microwave radiation.

The effect of 2.45 GHz microwave exposure on the in vivo distribution of pentobarbital is being studied by the use of ^{14}C-labeled sodium pentobarbital. Carbon-14 labeled anesthetic at the same dosage as previously employed in this investigation (22 mg/kg)is administered to Dutch rabbits who are then immediately subjected to either sham-irradiation (i.e. control animals) or exposure to 2.45 GHz CW microwave fields for predetermined periods of 15 or 30 min. or until the animal awakens (i.e. regains the righting reflex). At the end of this period the animal is sacrificed and tissue samples are obtained for the determination of ^{14}C-pentobarbital tissue concentrations by liquid scintillation counting. The results to date are summarized in Table 7 which lists the total ^{14}C activity in dpm/g tissue/μ Ci of administered ^{14}C-pentobarbital for the following tissues: brain, liver, kidney (cortex), adipose, muscle, serum, urine, and cerebrospinal fluid (CSF). These preliminary results do not indicate a clear effect of microwave exposure on pentobarbital distribution patterns in the Dutch rabbit. These data reflect total tissue ^{14}C-activity and it will thus be necessary to account for pentobarbital metabolism. The primary mode of pentobarbital elimination occurs via metabolic breakdown in the liver. The identification and quantitation of pentobarbital metabolites in tissue samples is currently being undertaken by the use of thin layer chromatography.

2. Gandhi, O.P. Conditions of Strongest Electromagnetic Power Deposition in Man and Animals. IEE Trans. MTT-23, 1021-1029 (1975).

TABLE 7

EFFECT OF 2.45 GHz MICROWAVE EXPOSURE ON THE IN VIVO DISTRIBUTION OF ^{14}C -LABELED PENTOBARBITAL (Mean±S.E.M.)

*All units dpm/g µCi TREATMENT	Brain	Liver	Kidney (Cortex)	Adipose	Muscle	Serum	Urine	CSF
Control n = 3 animals 15 min. uptake	$\bar{X}$ = 1216 S.E. = 162	$\bar{X}$ = 2768 S.E. = 181	$\bar{X}$ = 2343 S.E. = 681	$\bar{X}$ = 646 S.E. = 241	n = 2 $\bar{X}$ = 946 S.E. = 79	$\bar{X}$ = 1657 S.E. = 76	n = 2 $\bar{X}$ = 6944 S.E. = 4770	n = $\bar{X}$ = 923 S.E. = 426
Irradiated 10 mW/cm^2 n = 1 animal 15 min. uptake	980	432	4048	3457	--	1122	14,593	162
Control n = 2 animals 30 min. uptake	$\bar{X}$ = 907 S.E. = 109	$\bar{X}$ = 1848 S.E. = 72	$\bar{X}$ = 2450 S.E. = 113	$\bar{X}$ = 1174 S.E. = 49	$\bar{X}$ = 634 S.E. = 162	$\bar{X}$ = 1201 S.E. = 105	$\bar{X}$ = 10,859 S.E. = 8605	$\bar{X}$ = 342 S.E. = 21
Irradiated 10 mW/cm^2 n = 2 animals 30 min. uptake	$\bar{X}$ = 1681 S.E. = 377	$\bar{X}$ = 1986 S.E. = 299	$\bar{X}$ = 3498 S.E. = 255	$\bar{X}$ = 927 S.E. = 677	n = 1 789	$\bar{X}$ = 1603 S.E. = 216	$\bar{X}$ = 28,948 S.E. =13,173	n = 400
Irradiated 25 mW/cm^2 n = 2 animals 30 min. uptake	$\bar{X}$ = 1065 S.E. = 170	$\bar{X}$ = 2701 S.E. = 257	$\bar{X}$ = 2907 S.E. = 124	$\bar{X}$ = 1440 S.E. = 30	$\bar{X}$ = 808 S.E. = 209	$\bar{X}$ = 1399 S.E. = 228	$\bar{X}$ = 5300 S.E. = 3444	$\bar{X}$ = 441 S.E. = 140
Control n = 1 animal 50 min. uptake	1546	2242	2410	1260	746	1234	179,822	352
Irradiated 10 mW/cm^2 n = 2 animals Imm. post-awake	$\bar{X}$ = 285 S.E. = 286	$\bar{X}$ = 1309 S.E. = 605	$\bar{X}$ = 1592 S.E. = 1087	$\bar{X}$ = 275 S.E. = 61	n = 1 475	$\bar{X}$ = 870 S.E. = 42	$\bar{X}$ = 48,261 S.E. =25,376	$\bar{X}$ = 244 S.E. = 23
Irradiated 25 mW/cm^2 n = 2 animals Imm. post-awake	$\bar{X}$ = 1392 S.E. = 49	$\bar{X}$ = 2700 S.E. = 267	$\bar{X}$ = 3797 S.E. = 457	$\bar{X}$ = 1232 S.E. = 263	$\bar{X}$ = 824 S.E. = 54	$\bar{X}$ = 1358 S.E. = 221	$\bar{X}$ = 5757 S.E. = 5023	$\bar{X}$ = 896 S.E. = 401

VI EFFECT OF MICROWAVE EXPOSURE ON TONIC IMMOBILIZATION IN THE DUTCH RABBIT

In an attempt to ascertain the sensitivity of Dutch rabbits to microwave stress in an unanesthetized state for comparison with the results obtained in the study of microwave effects on pentobarbital-induced sleeping time, the use of tonic (reflexive) immobilization has been investigated. This technique, which is also referred to as "animal hypnosis", has been described in detail by Klemm[3]. Rabbits are inverted and placed on their backs in a box consisting of a base and two sides spaced 10 cm apart such that mild pressure is exerted on the animal's rib cage. Proper immobilization results in the induction of a hypnotic-like state in the rabbit, characterized by a relaxed, motionless attitude with slow, deep respiration. The rabbit will remain in this state for periods of up to 2 hr if not disturbed. This method of immobilization was used with a group of 8 rabbits and the results of the first 5 trials are summarized in Table 8. Although these results are preliminary, it appears that individual animals vary in their durations of tonic immobilization, and repeated application of the technique seems to lengthen the duration of the effect. A trial experiment has been conducted to determine the usefulness of this method in the investigation of microwave-induced arousal. In this case rabbits were tonically immobilized in an anechoic chamber at the Department of Microwave Research, WRAIR, Silver Spring, Maryland and following a 5 min waiting period the animals were exposed to 25 mW/cm^2 CW microwaves at a frequency of 2.45 GHz and the duration of the immobilized state was determined by observing the animals using closed circuit television. Animal number 26, who had a mean immobilization time of 25 min during sham exposure conditions, remained immobilized for 3 min in each of two exposures to the microwave field. Animal number 12, who had a mean immobilization duration of 51 min under sham exposure conditions, had a mean immobilization duration of 12 min in two trials while exposed to the 25 mW/cm^2 field. These data are obviously not sufficient for a statistical evaluation of the effect of microwave exposure on the duration of immobilization, but it is indicated that this technique is potentially useful for the study of such effects of exposure on the Dutch rabbit. Additional investigation of this phenomenon will be undertaken in the near future.

VII IN VIVO AND IN VITRO EFFECTS OF MICROWAVE RADIATION ON SERUM PROTEINS

The results of studies of the in vivo effects of microwave exposure on Dutch rabbit serum proteins were described in Annual Report Number 2 (June 1974). These studies involved the use of acrylamide gel electrophoresis to detect alterations in rabbit serum protein distributions following exposure of the experimental animals to 2.45 and 1.7 GHz microwave fields. Although there were suggestive changes in the serum protein distributions, primarily an increase in proteins which migrate in the α-region, the electrophoretic technique did not have adequate resolving power to provide a definite indication of the nature of the microwave effect. During the past contract year, methods for the analysis of such changes have been developed, including the assembly and testing of a microwave waveguide irradiation apparatus for the in vitro exposure of serum proteins and the development of analytical iso-electric focusing electrophoresis techniques for high resolution serum protein assays.

3. Klemm, W. R. A Method to Encourage Extensive Study of Animal Hypnotic Behavior. J. Exptl. Analysis of Behavior, 9, 63 (1966).

TABLE 8 TONIC IMMOBILIZATION DURATIONS

RABBIT NUMBER	IMMOBILIZATION TIME (MIN.) TRIAL NUMBER					MEAN (± S.E.M.) IMMOBILIZATION DURATION (min.)
	1	2	3	4	5	
12	44,8,31	47	58	60	108	51 ± 11.6
22	10,10	20	13,50	26,53	6,58	27 ± 6.9
19	2,3	15,3	13	17,16	-	10 ± 2.6
15	16	41	9,5,17	20	-	18 ± 5.1
23	55	30	60	60	15,12	39 ± 9.2
26	32	20,12	27,7	60	21,23	25 ± 5.7
11	15	13,13	5,26	7,3,5	-	11 ± 2.7
13	53	3,13	27,26	60	-	30 ± 9.1
TRIAL MEAN IMMOB. DURATION (±S.E.M.)(min)	23.3±5.5	19.2±4	24.5±5.1	32.3±7	34.7±13.8	26.0±2.9

The microwave waveguide apparatus utilizes a Hewlett-Packard (HP) Model 692-C sweep oscillator which operates from 2 to 4 GHz and a HP 491-C microwave amplifier with a rated output of one watt. The CW microwave field generated by this apparatus provides up to 80 mW/cm^2 at the center of the S-band (WR284) waveguide where the serum samples will be irradiated. The microwave intensity is controlled by a leveling loop using HP power meters and the radiation is delivered to the waveguide through an isolator. Two multi-hole directional couplers are used to measure the incident and reflected power; the waveguide is terminated with a matched load. The sample irradiation cell consists of a 1 cm O.D. glass tube inserted perpendicularly through the center of the waveguide at the point of maximum interaction with the E-field of the microwave radiation. Absorbed power in the sample will be determined by measuring the return loss and insertion loss.

The experimental conditions for optimal resolution of rabbit serum proteins using the iso-electric focusing electrophoresis technique have been determined and a microelectrode pH probe has been calibrated for the determination of pH gradients in the focusing gel. Preliminary runs with this apparatus have been completed and the high resolving power of this technique for serum protein determinations has been verified.

PUBLICATIONS

Publications that have resulted from support provided by the U. S. Army Medical Research and Development Command under Contract No. DADA 17-72-C-2144 during the past contract year are as follows:

1. Wangemann, R. T. and S. F. Cleary. The In Vivo Effects of 2.45 GHz Microwave Radiation on Rabbit Serum Components. Rad. and Environ. Biophysics (in press).

2. Cleary, S. F. and R. T. Wangemann. Effect of Microwave Radiation on Pentobarbital-induced Sleeping Time. Proceedings of the 1975 Annual Meeting, United States National Committee-International Union of Radio Science (in press).

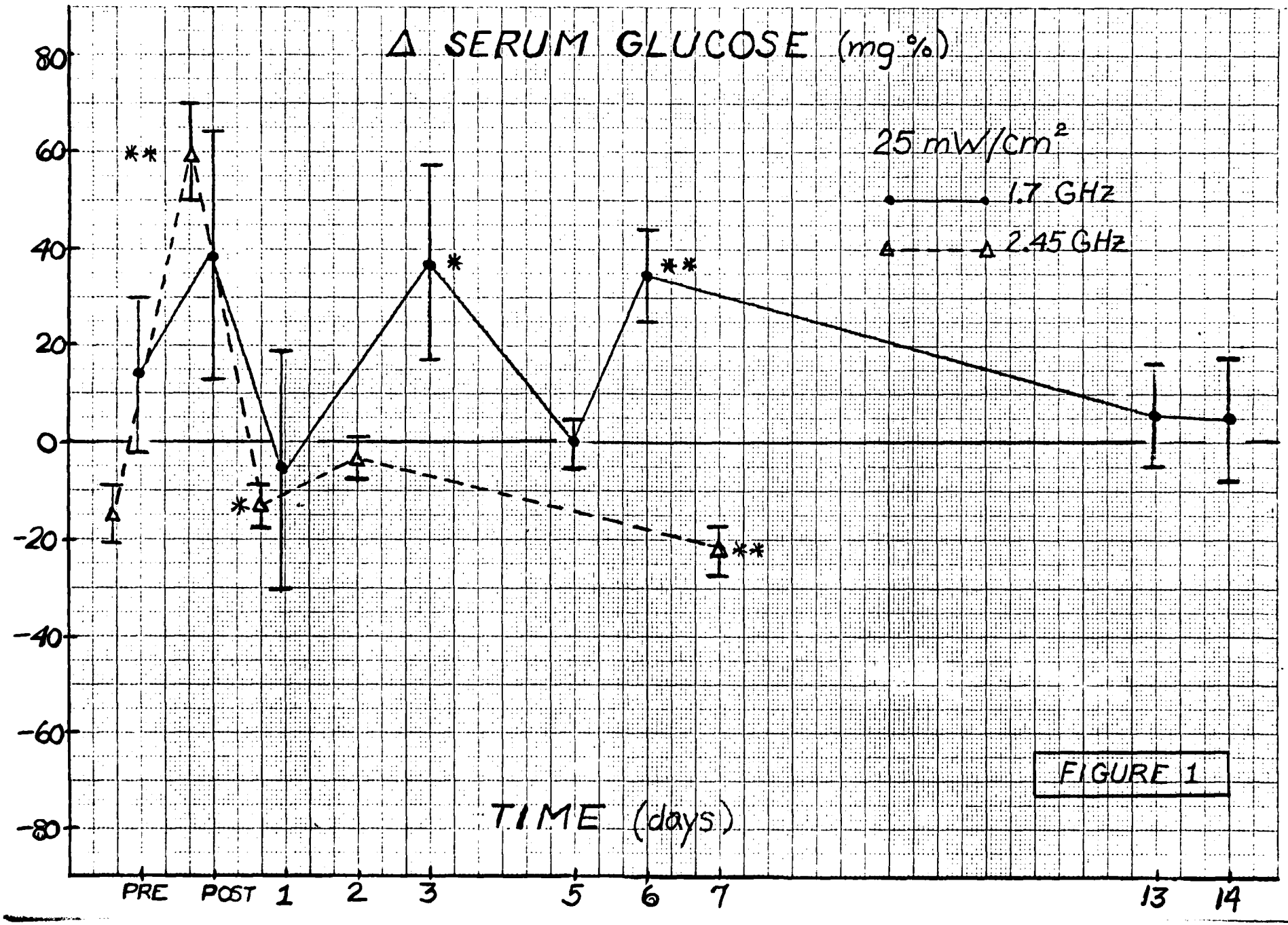
Δ SERUM GLUCOSE (mg %)
25 mW/cm²
1.7 GHz
2.45 GHz
80
60
40
20
0
-20
-40
-60
-80
PRE
POST
1
2
3
5
6
7
13
14
TIME (days)

FIGURE 1

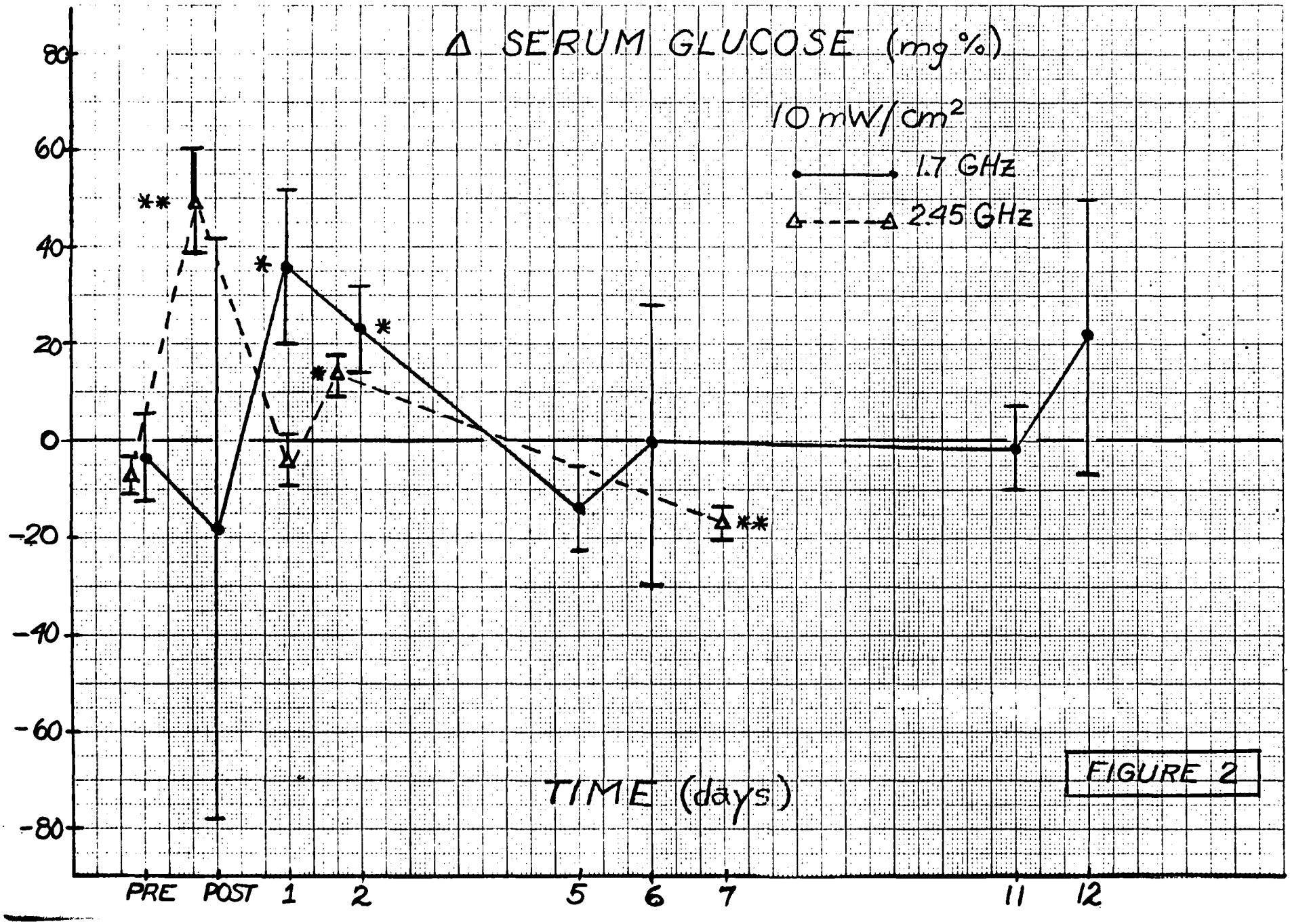
Δ SERUM GLUCOSE (mg %)
10 mW/cm²
1.7 GHz
2.45 GHz
80
60
40
20
0
-20
-40
-60
-80
PRE
POST
1
2
5
6
7
11
12
TIME (days)

FIGURE 2

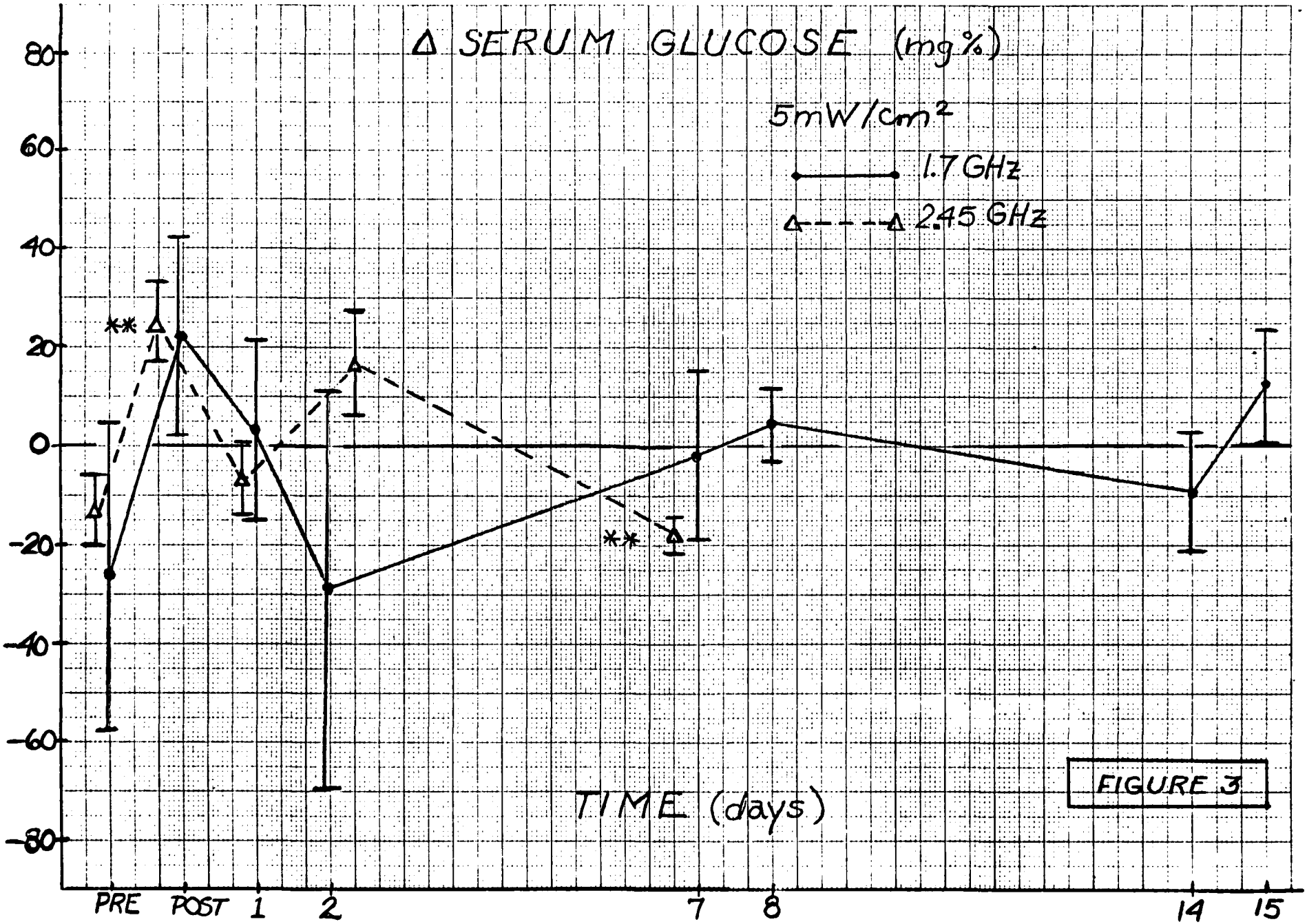
Δ SERUM GLUCOSE (mg%)
5mW/cm²
1.7 GHz
2.45 GHz
80
60
40
20
0
-20
-40
-60
-80
**
**
PRE
POST
1
2
7
8
14
15
TIME (days)

FIGURE 3

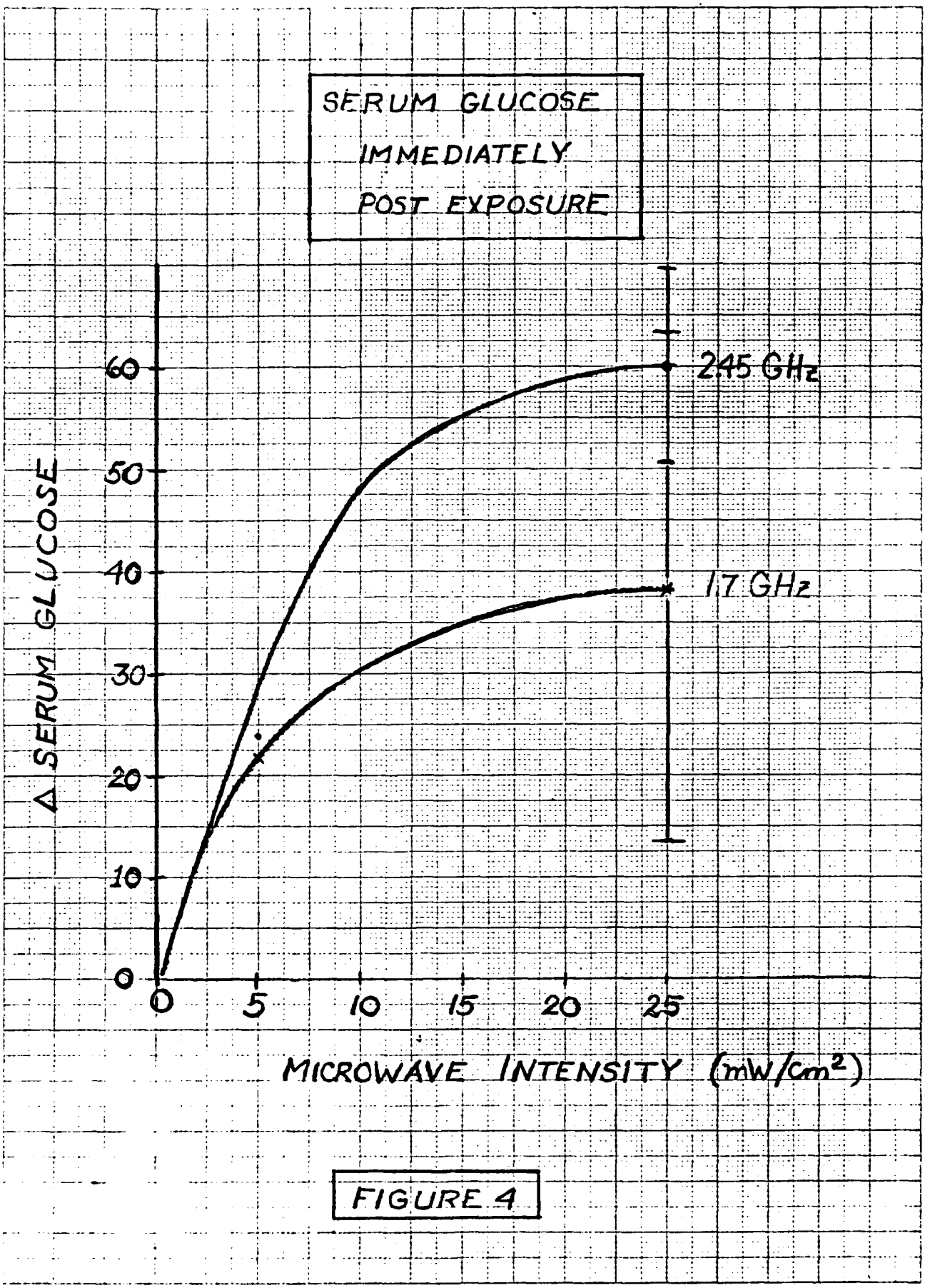

FIGURE 4

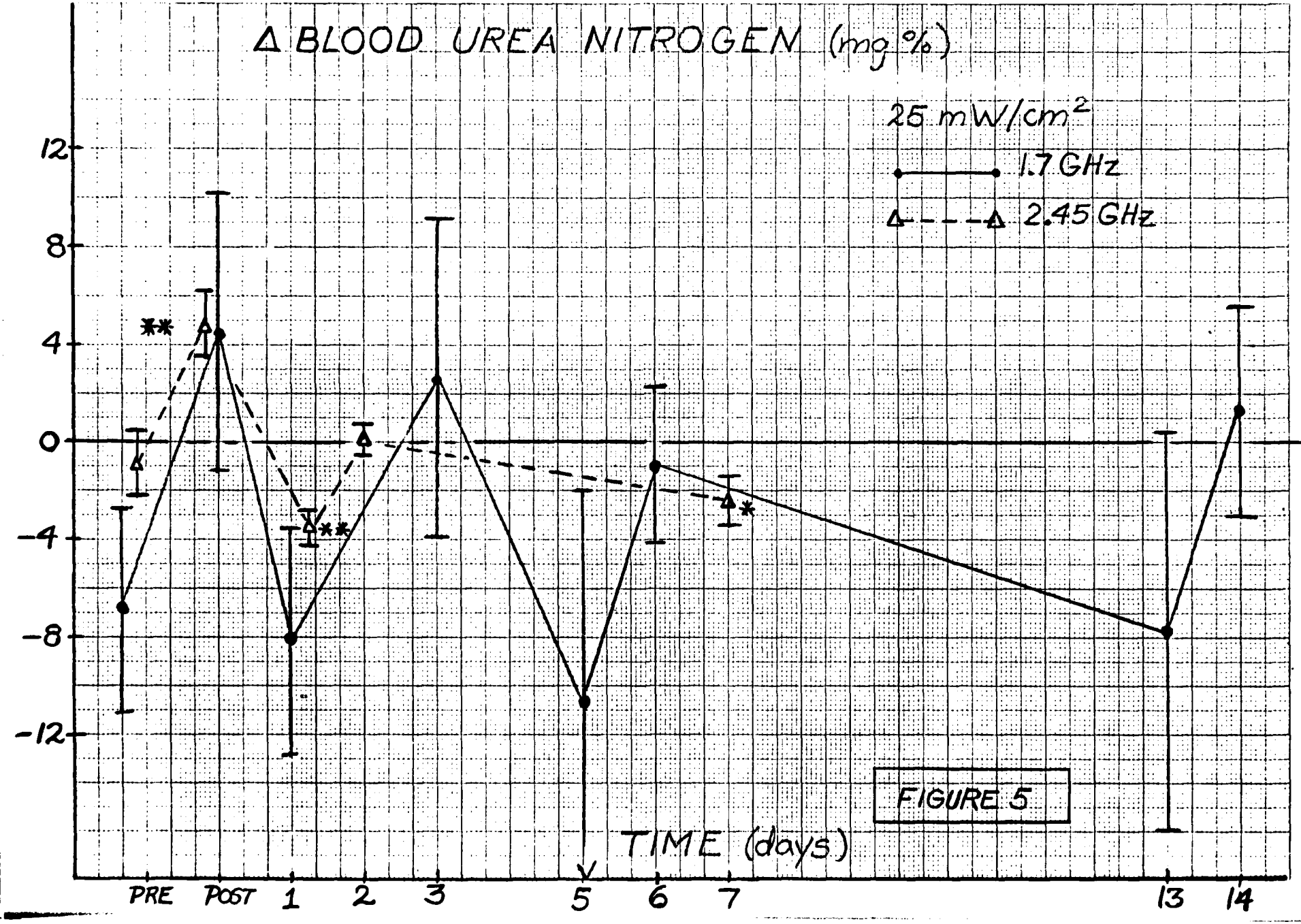

FIGURE 5

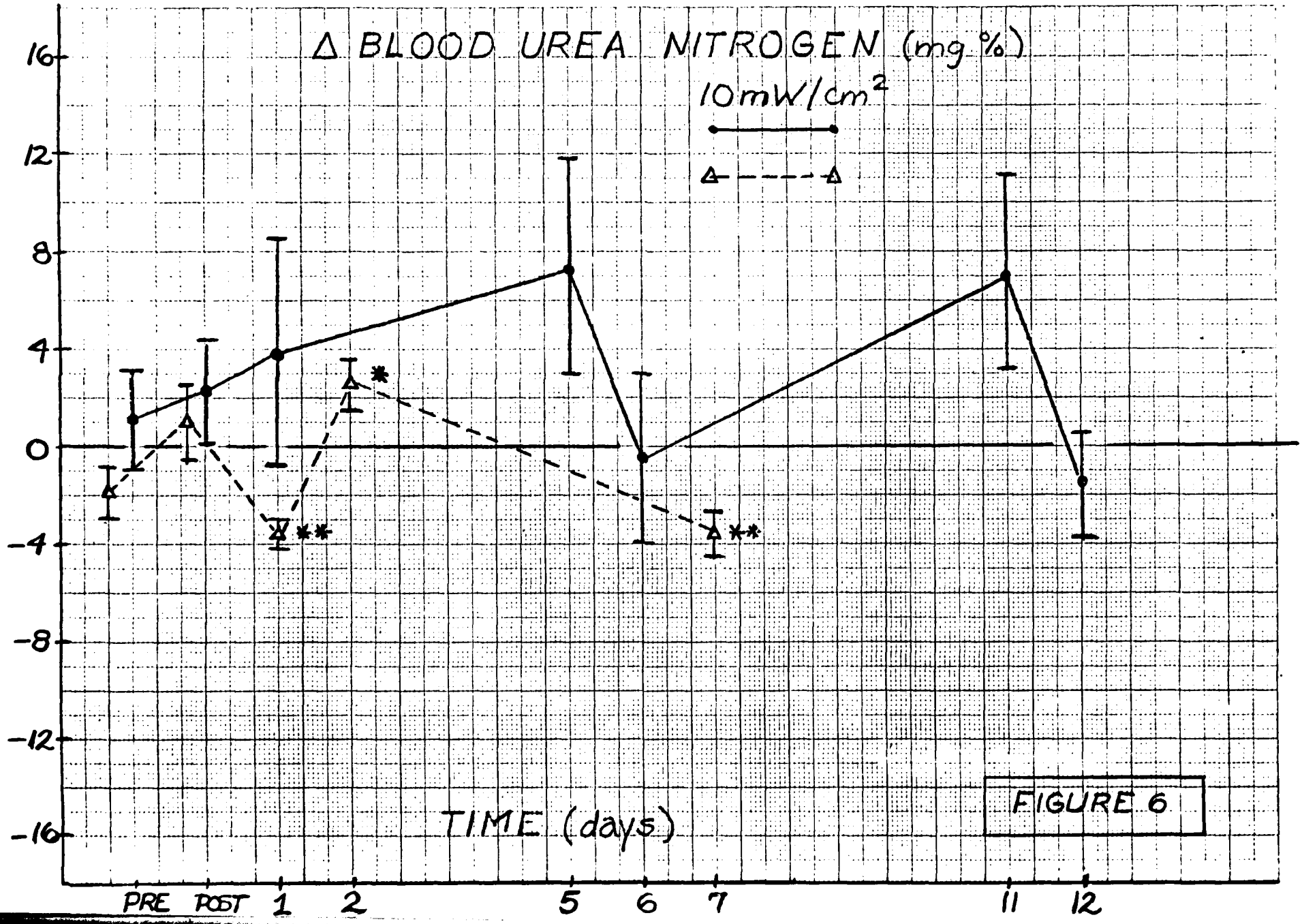
Δ BLOOD UREA NITROGEN (mg %)
10mW/cm²
16
12
8
4
0
-4
-8
-12
-16
*
**
**
TIME (days)
PRE
POST
1
2
5
6
7
11
12

FIGURE 6

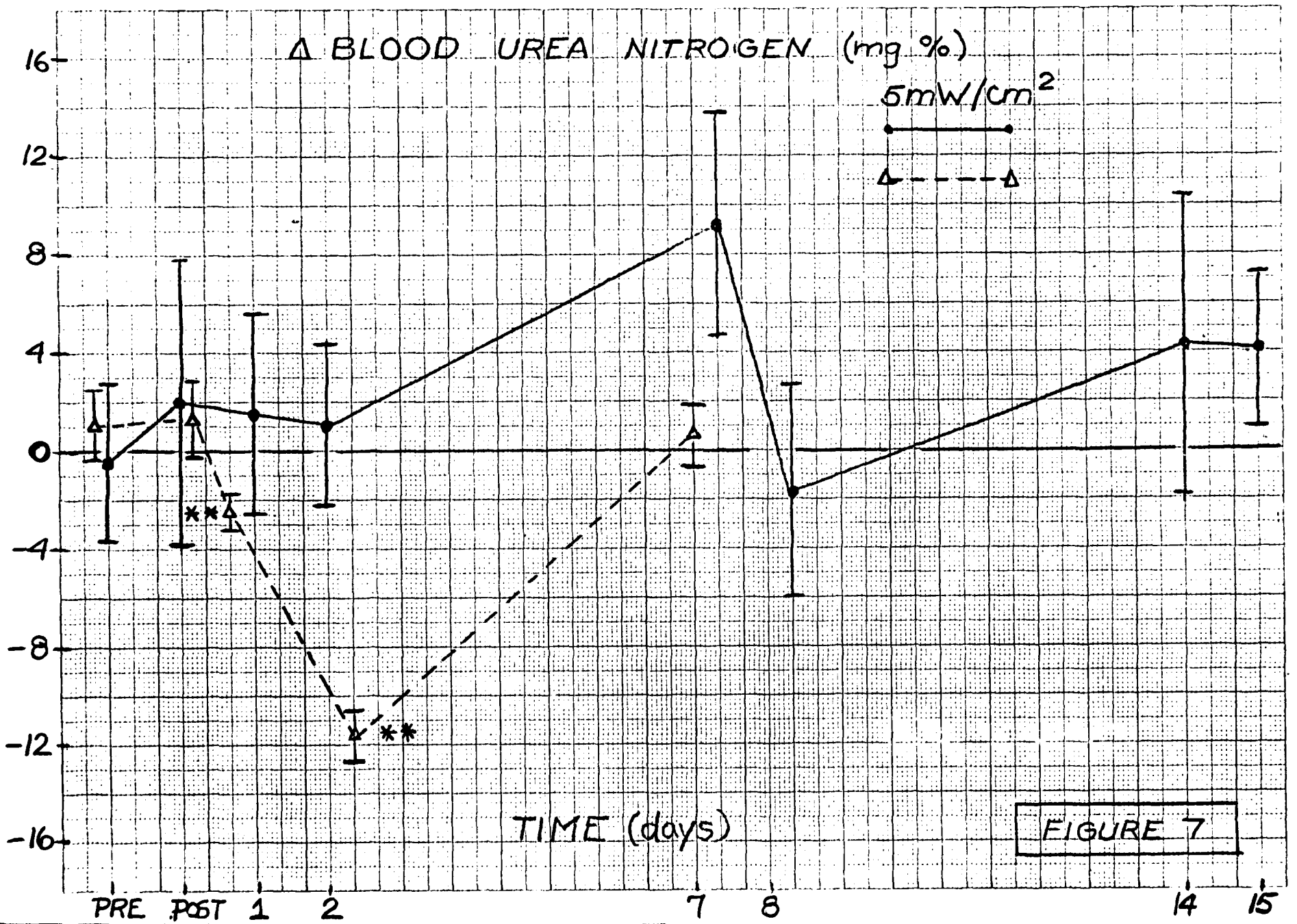
Δ BLOOD UREA NITROGEN (mg %)
5mW/cm²
16
12
8
4
0
-4
-8
-12
-16
PRE
POST
1
2
7
8
14
15
TIME (days)

FIGURE 7

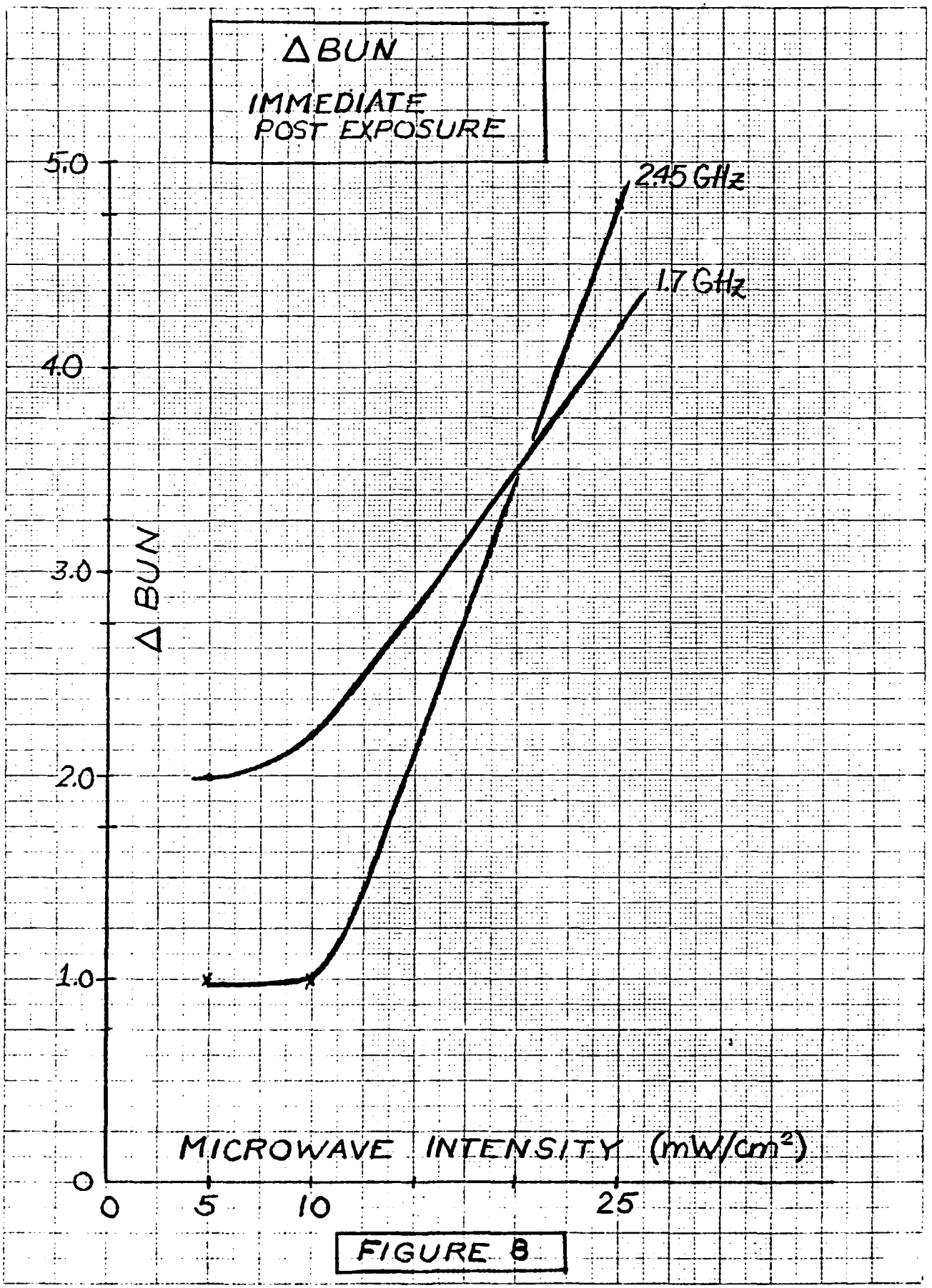

FIGURE 8

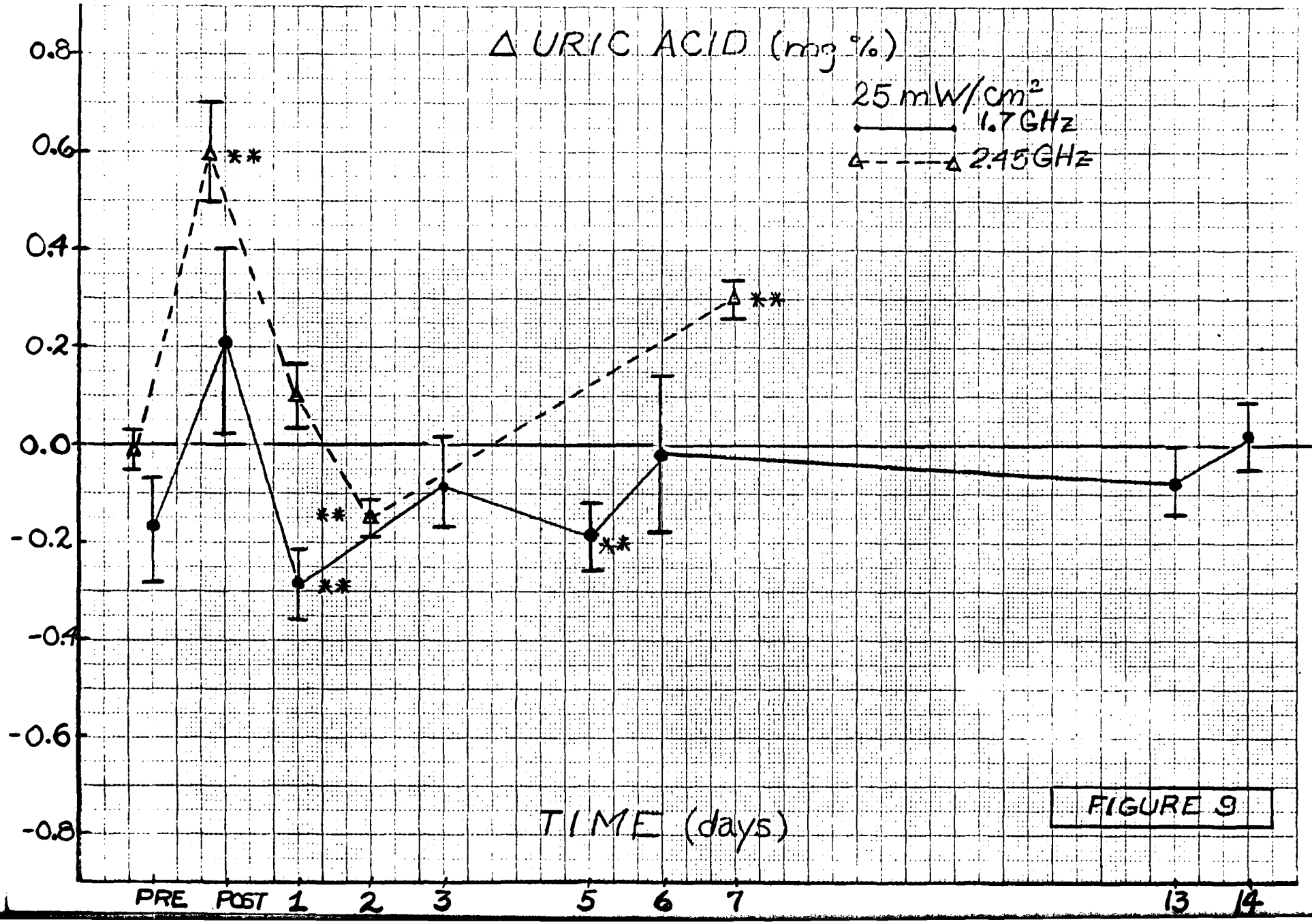
Δ URIC ACID (mg %)
25 mW/cm²
1.7 GHz
2.45 GHz
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
**
**
**
**
**
PRE
POST
1
2
3
5
6
7
13
14
TIME (days)

FIGURE 9

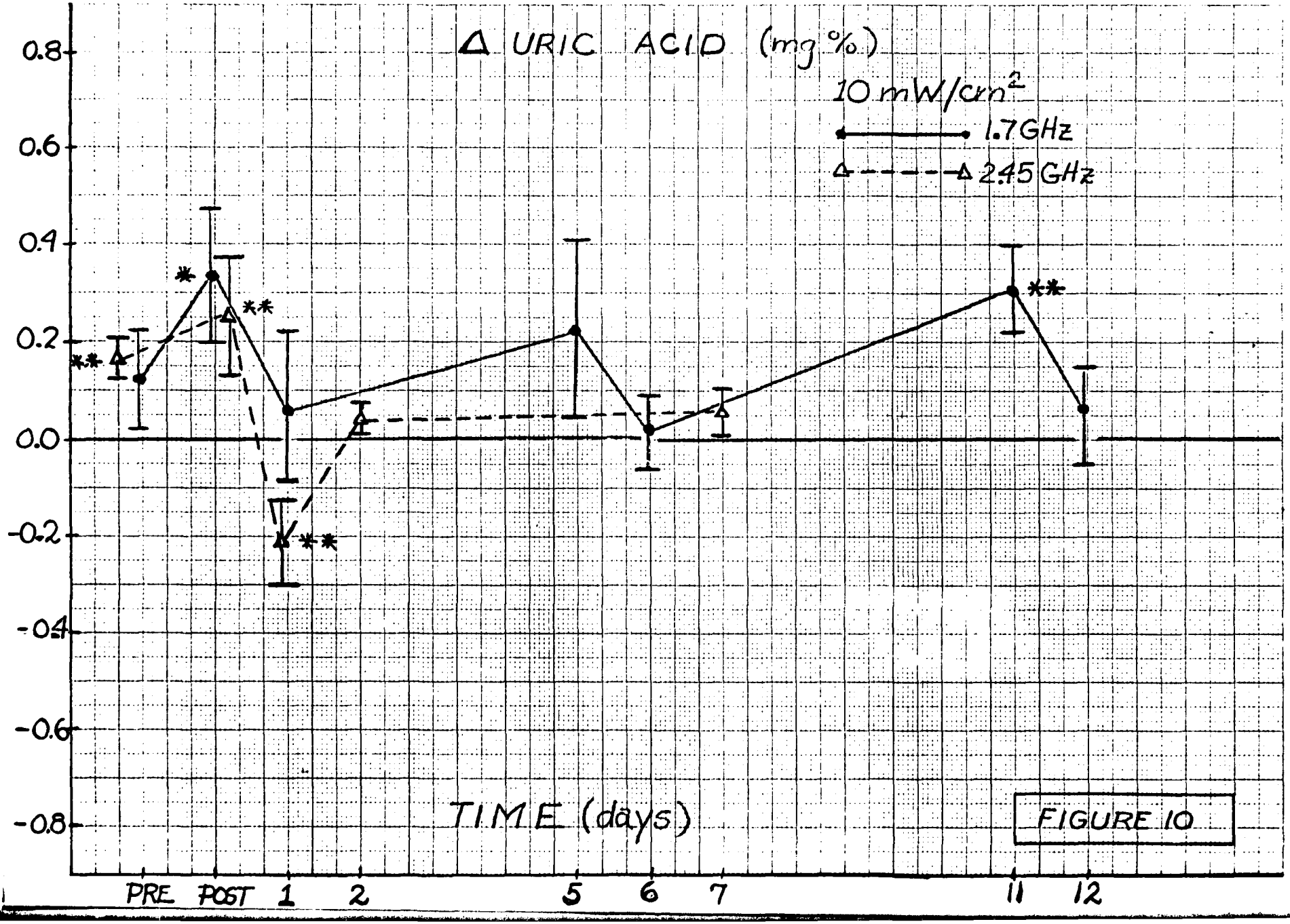
Δ URIC ACID (mg %)
10 mW/cm²
1.7 GHz
2.45 GHz
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
TIME (days)
PRE
POST
1
2
5
6
7
11
12

FIGURE 10

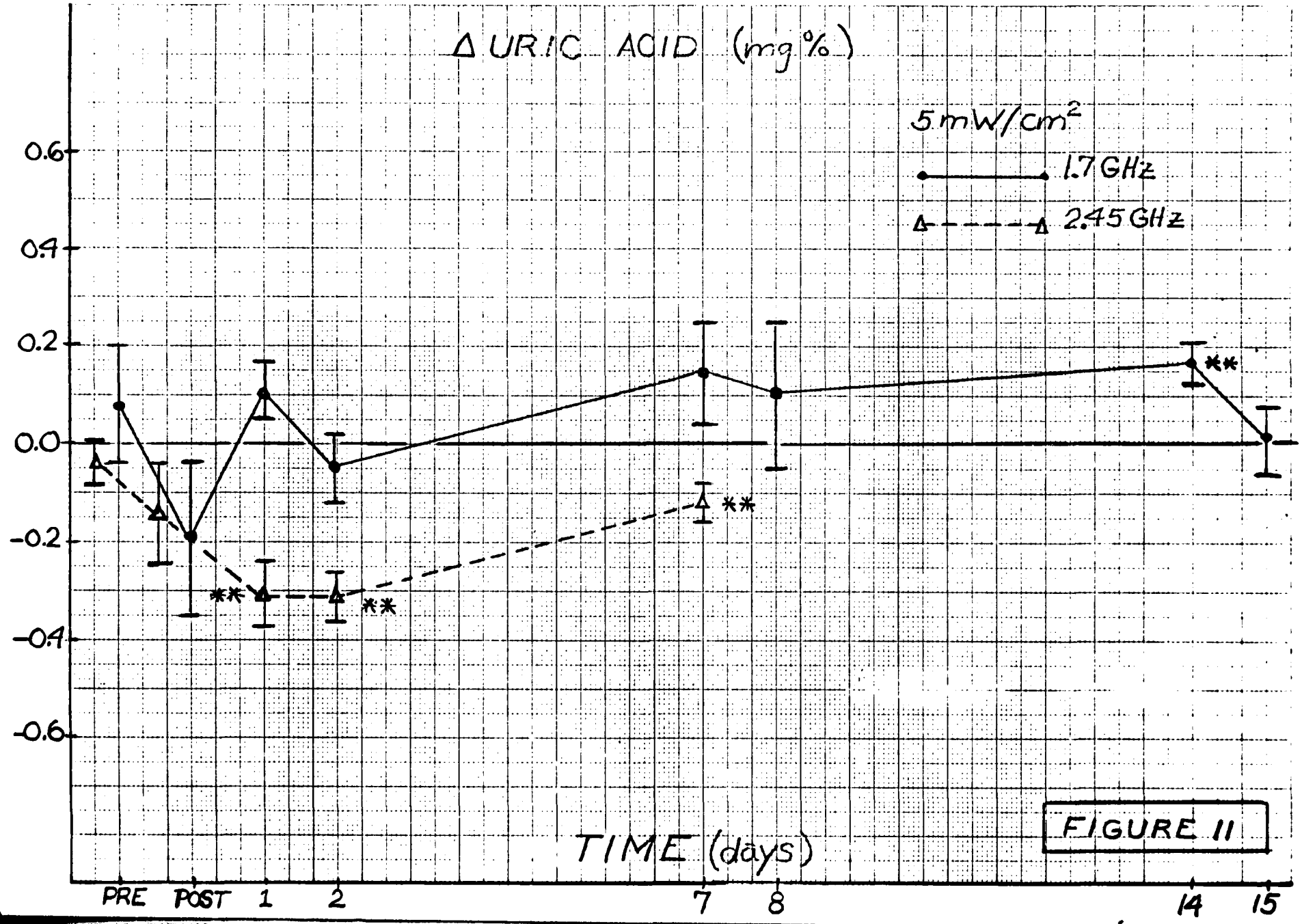
Δ URIC ACID (mg%)
5 mW/cm²
1.7 GHz
2.45 GHz
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
**
**
**
PRE
POST
1
2
7
8
14
15
TIME (days)

FIGURE 11

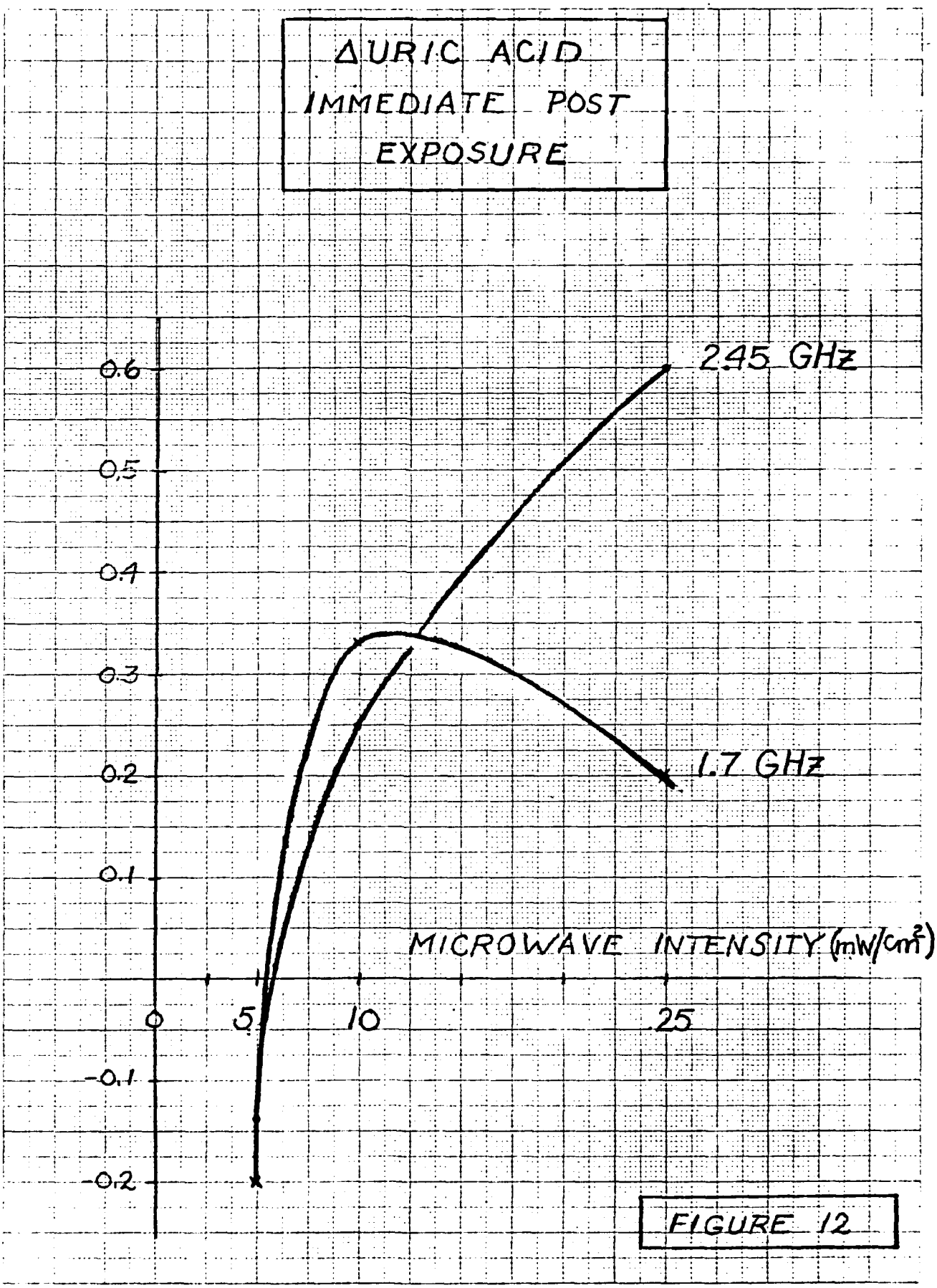

FIGURE 12

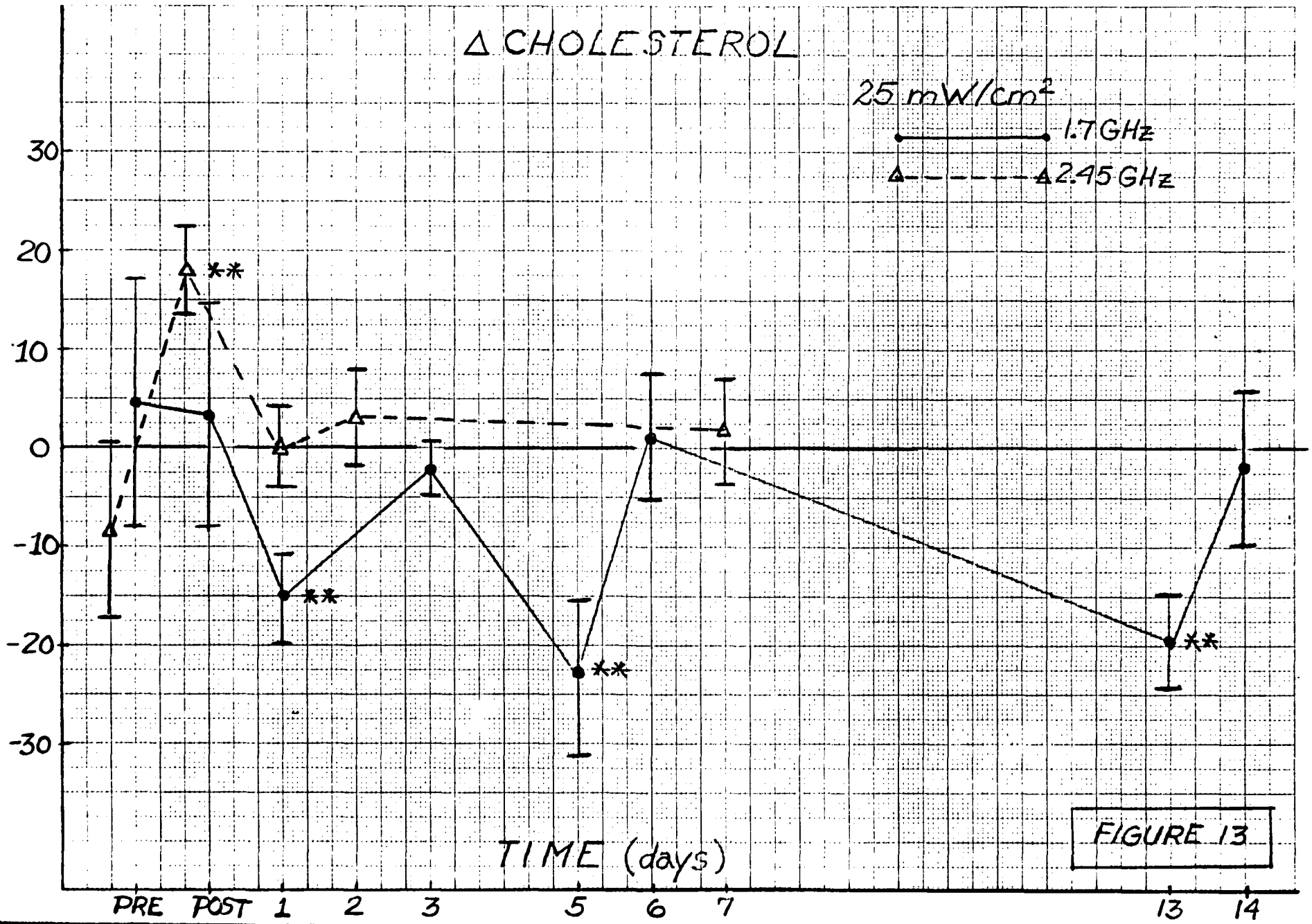
Δ CHOLESTEROL
25 mW/cm²
1.7 GHz
2.45 GHz
30
20
10
0
-10
-20
-30
**
**
**
**
PRE
POST
1
2
3
5
6
7
13
14
TIME (days)

FIGURE 13

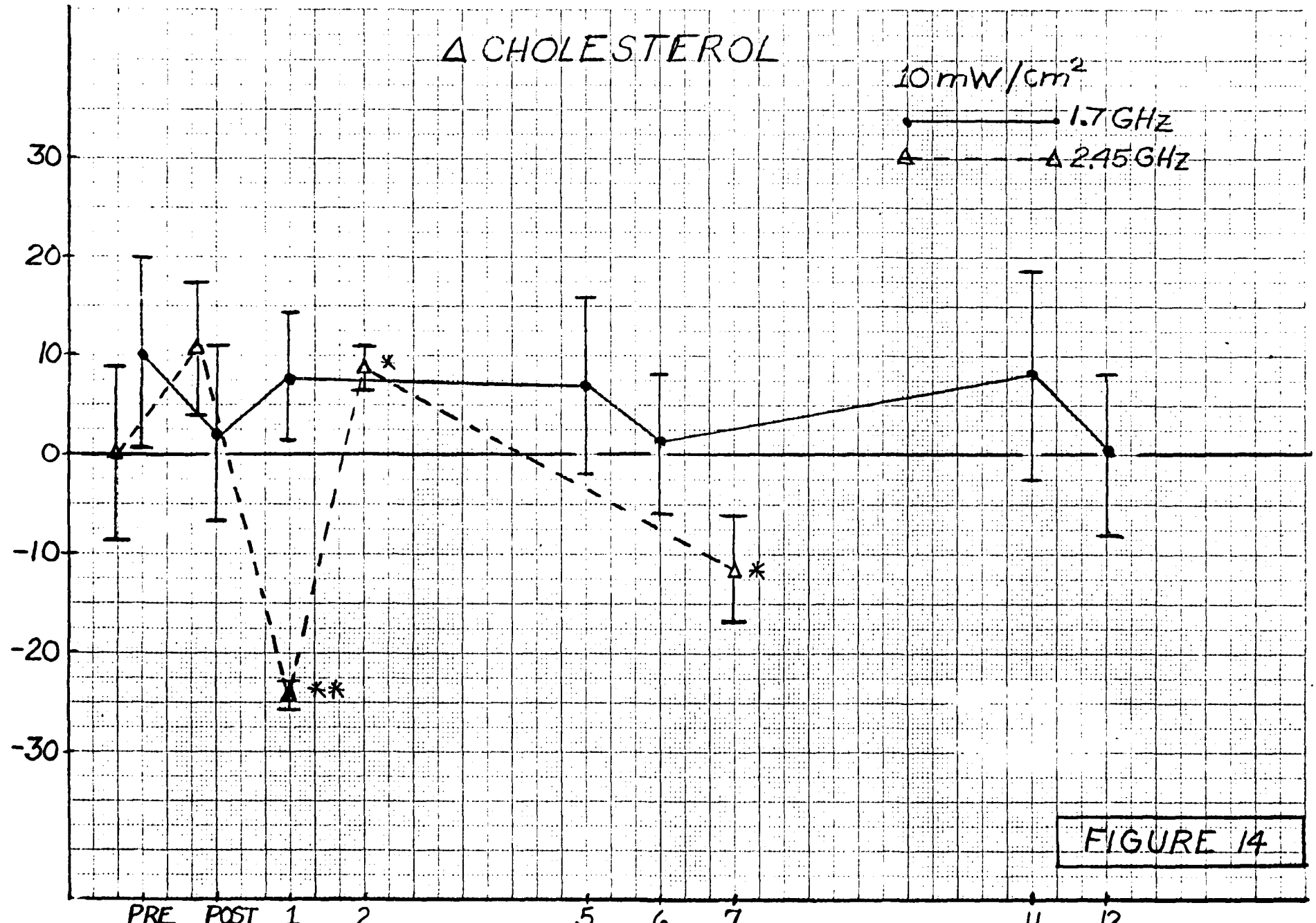
Δ CHOLESTEROL
10 mW/cm²
1.7 GHz
2.45 GHz
30
20
10
0
-10
-20
-30
PRE
POST
1
2
5
6
7
11
12
*
**

FIGURE 14

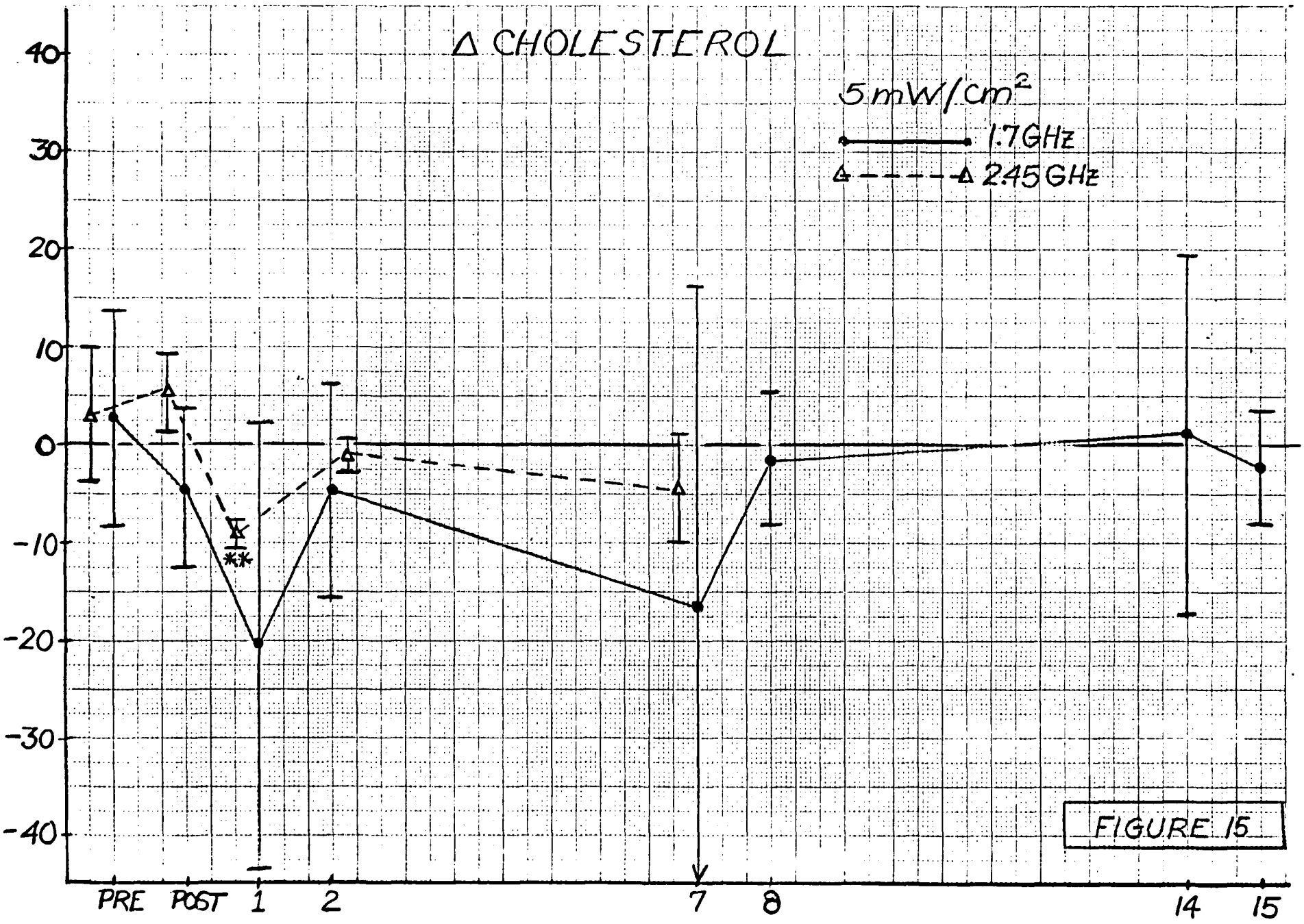
Δ CHOLESTEROL
5 mW/cm²
1.7 GHz
2.45 GHz
40
30
20
10
0
-10
-20
-30
-40
**
PRE
POST
1
2
7
8
14
15

FIGURE 15

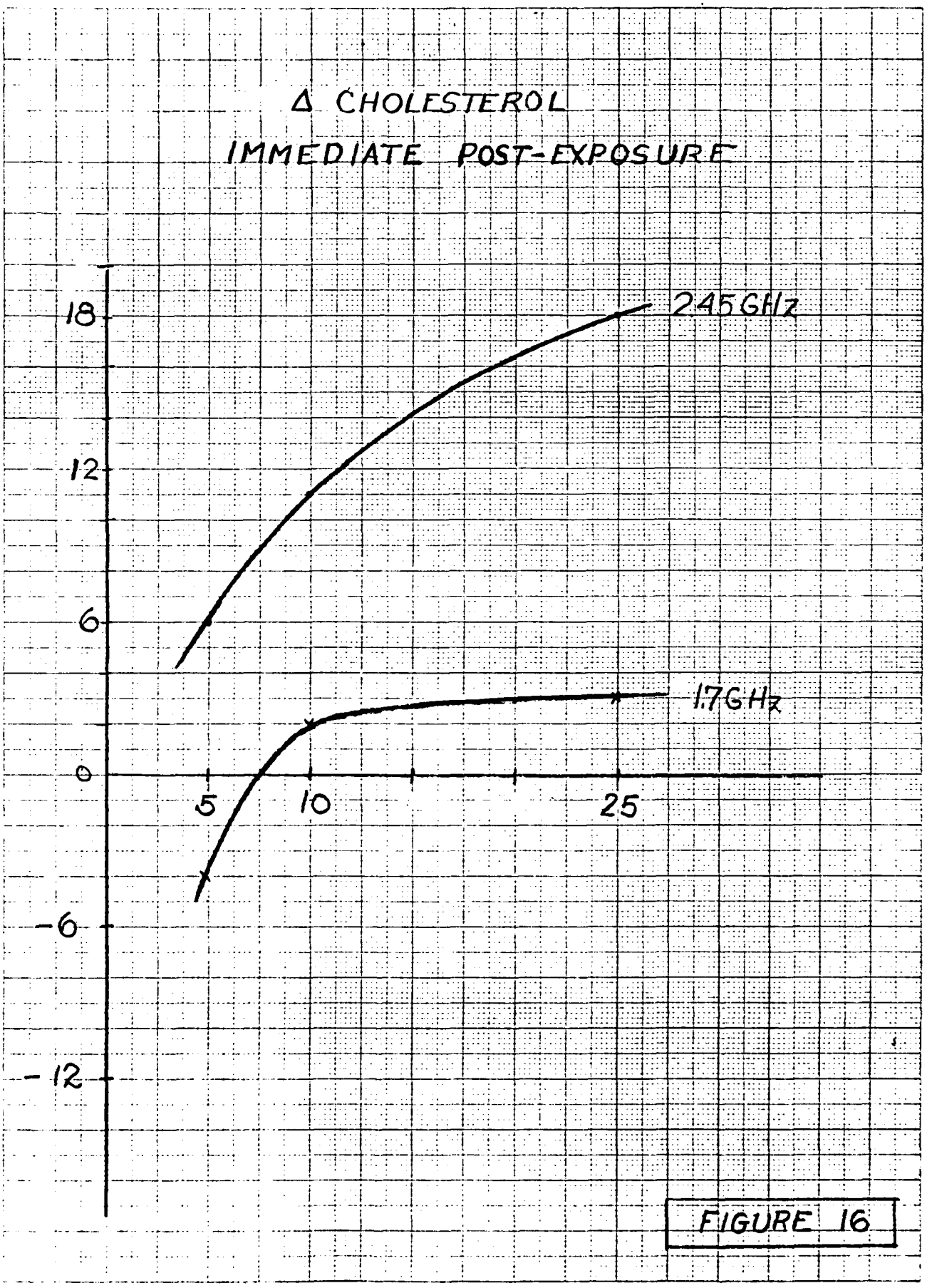

FIGURE 16

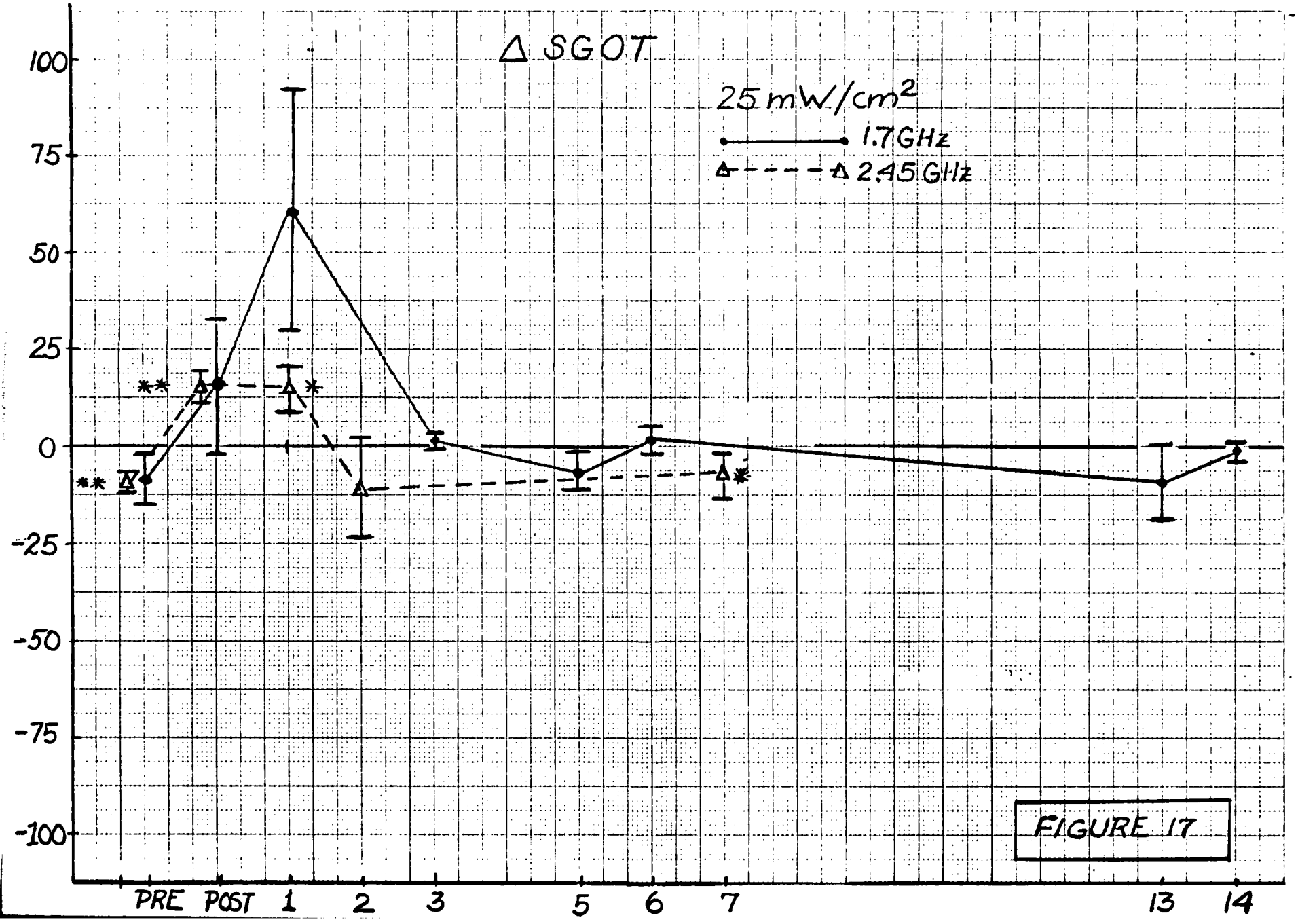
Δ SGOT
25 mW/cm²
1.7 GHz
2.45 GHz
100
75
50
25
0
-25
-50
-75
-100
PRE
POST
1
2
3
5
6
7
13
14

FIGURE 17

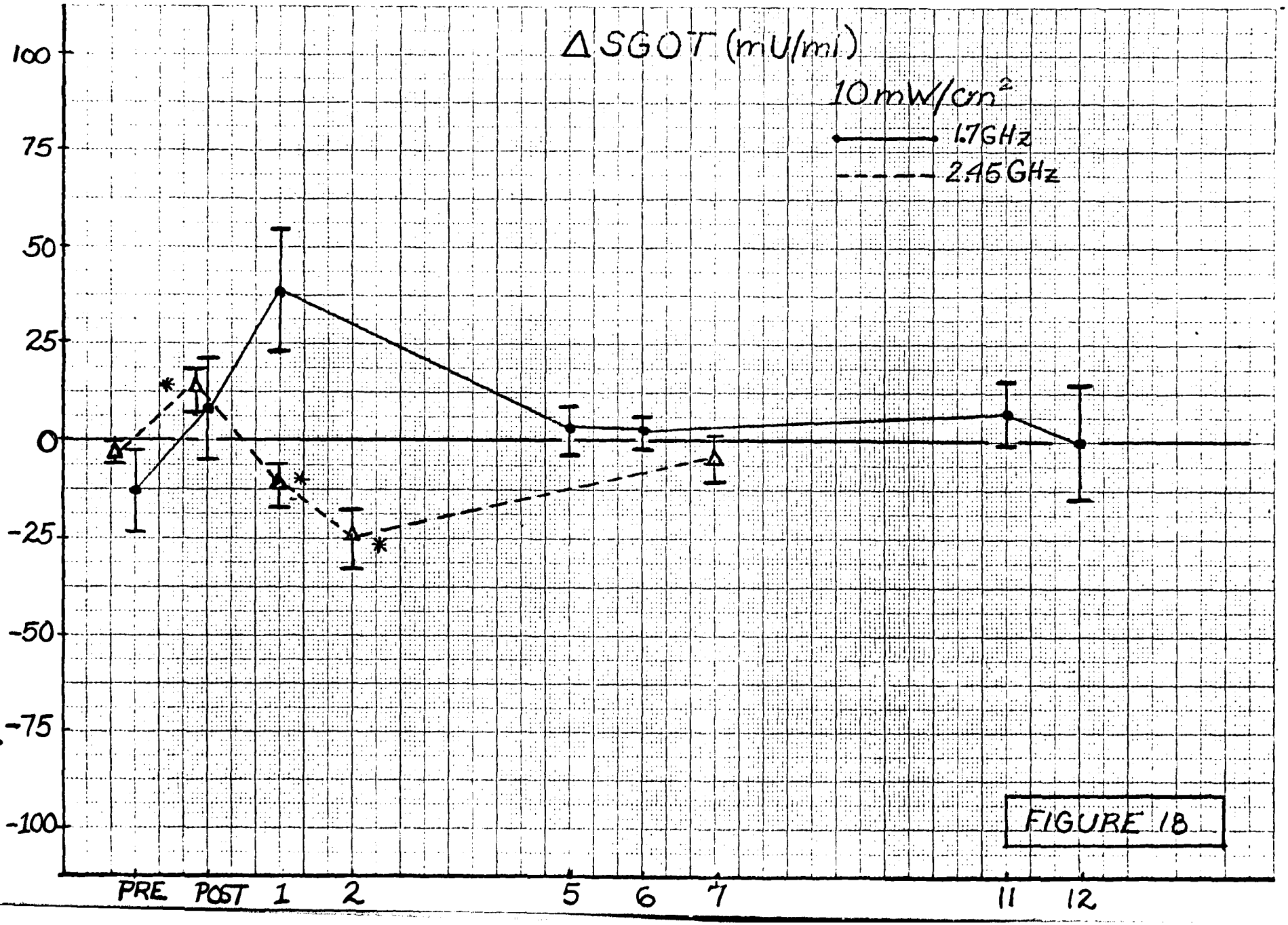
ΔSGOT (mU/ml)
10 mW/cm²
1.7 GHz
2.45 GHz
100
75
50
25
0
-25
-50
-75
-100
PRE
POST
1
2
5
6
7
11
12

FIGURE 18

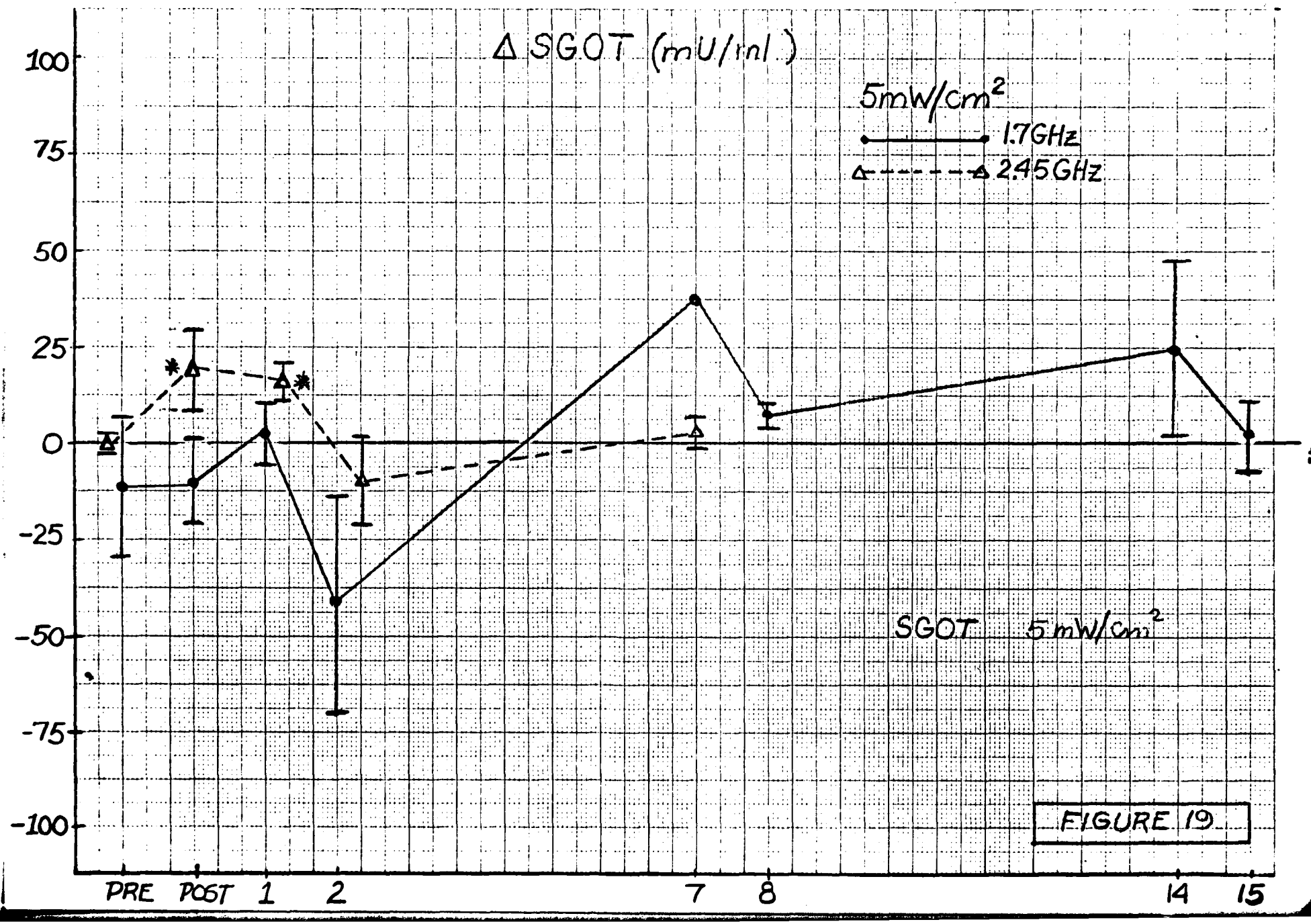
Δ SGOT (mU/ml)
5mW/cm²
1.7GHz
2.45GHz
100
75
50
25
0
-25
-50
-75
-100
PRE
POST
1
2
7
8
14
15
SGOT 5 mW/cm²

FIGURE 19

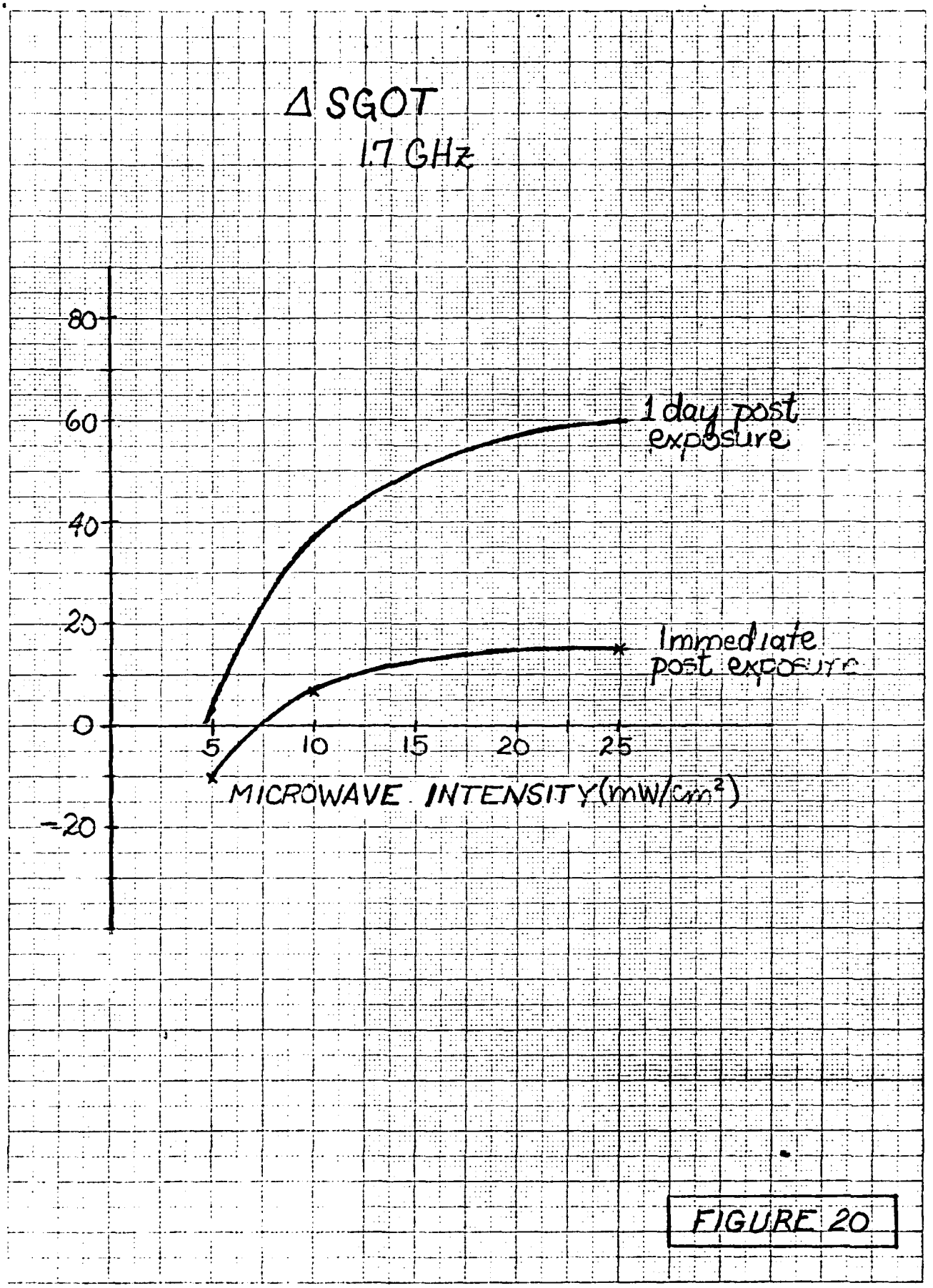

FIGURE 20

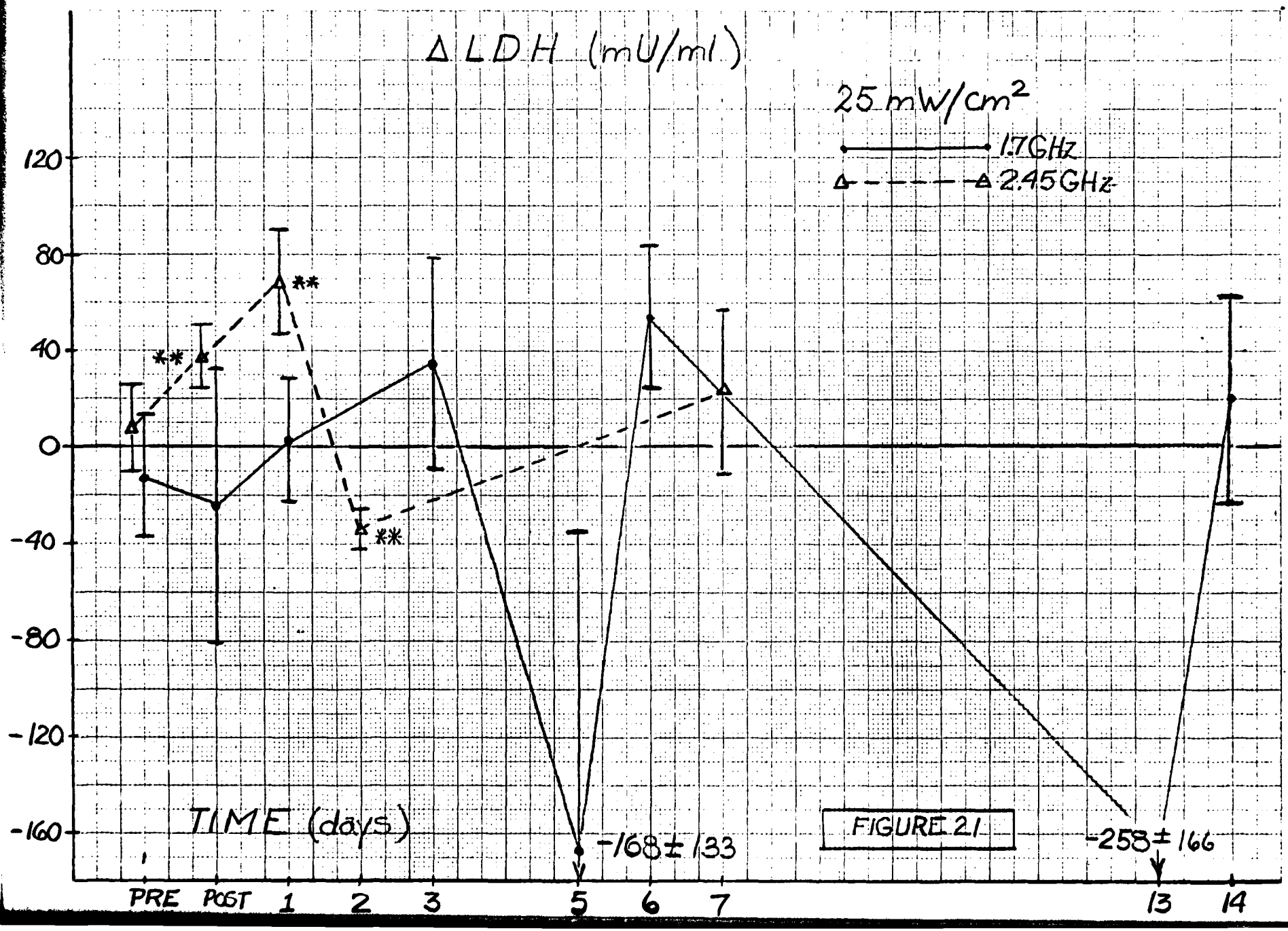
ΔLDH (mU/ml)
25 mW/cm²
1.7GHz
2.45GHz
120
80
40
0
-40
-80
-120
-160
**
**
**
-168 ± 133
-258 ± 166
TIME (days)
PRE
POST
1
2
3
5
6
7
13
14

FIGURE 21

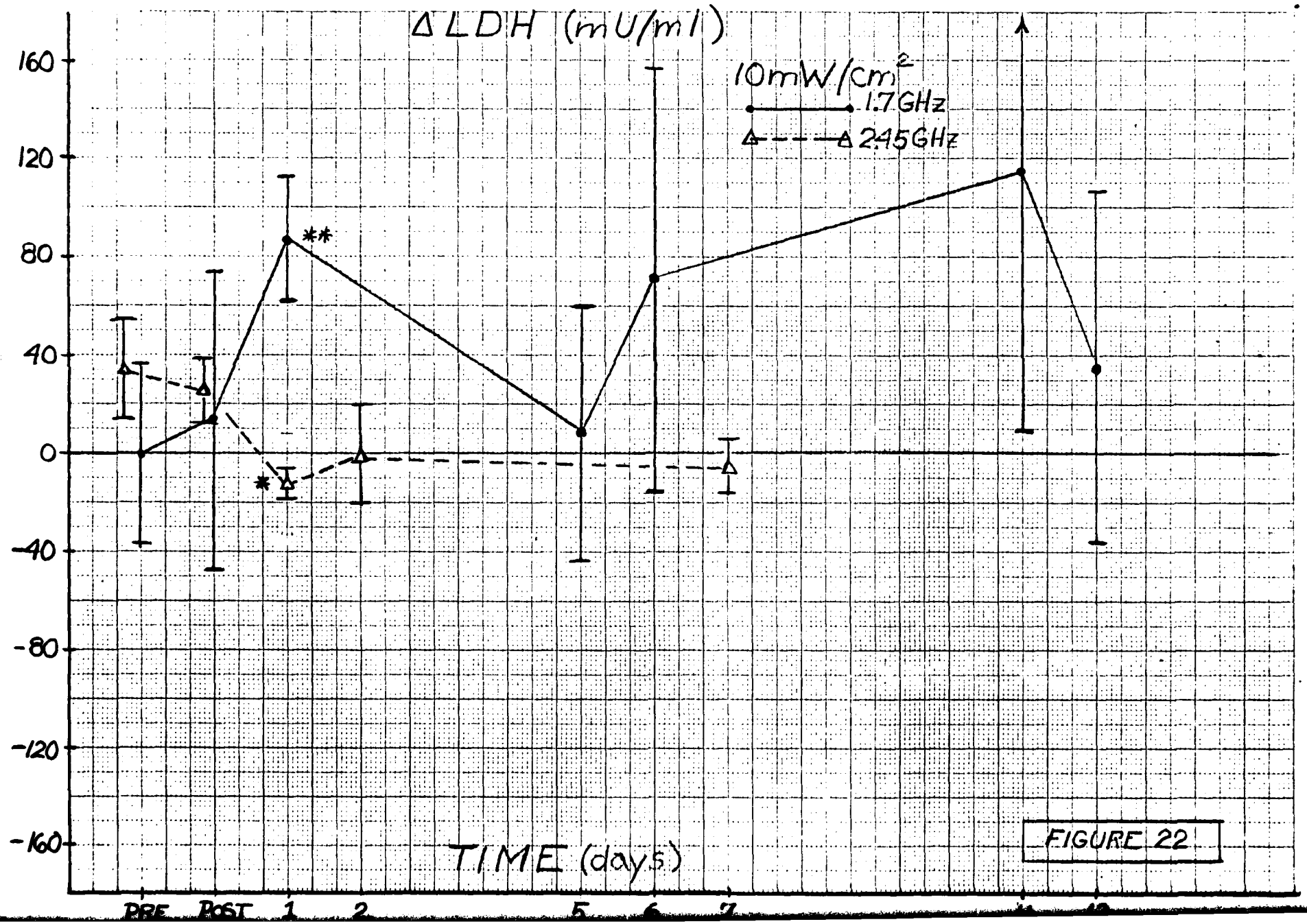
ΔLDH (mU/ml)
160
120
80
40
0
-40
-80
-120
-160
10mW/cm²
1.7GHz
2.45GHz
**
*
TIME (days)
PRE
POST
1
2
5
6
7

FIGURE 22

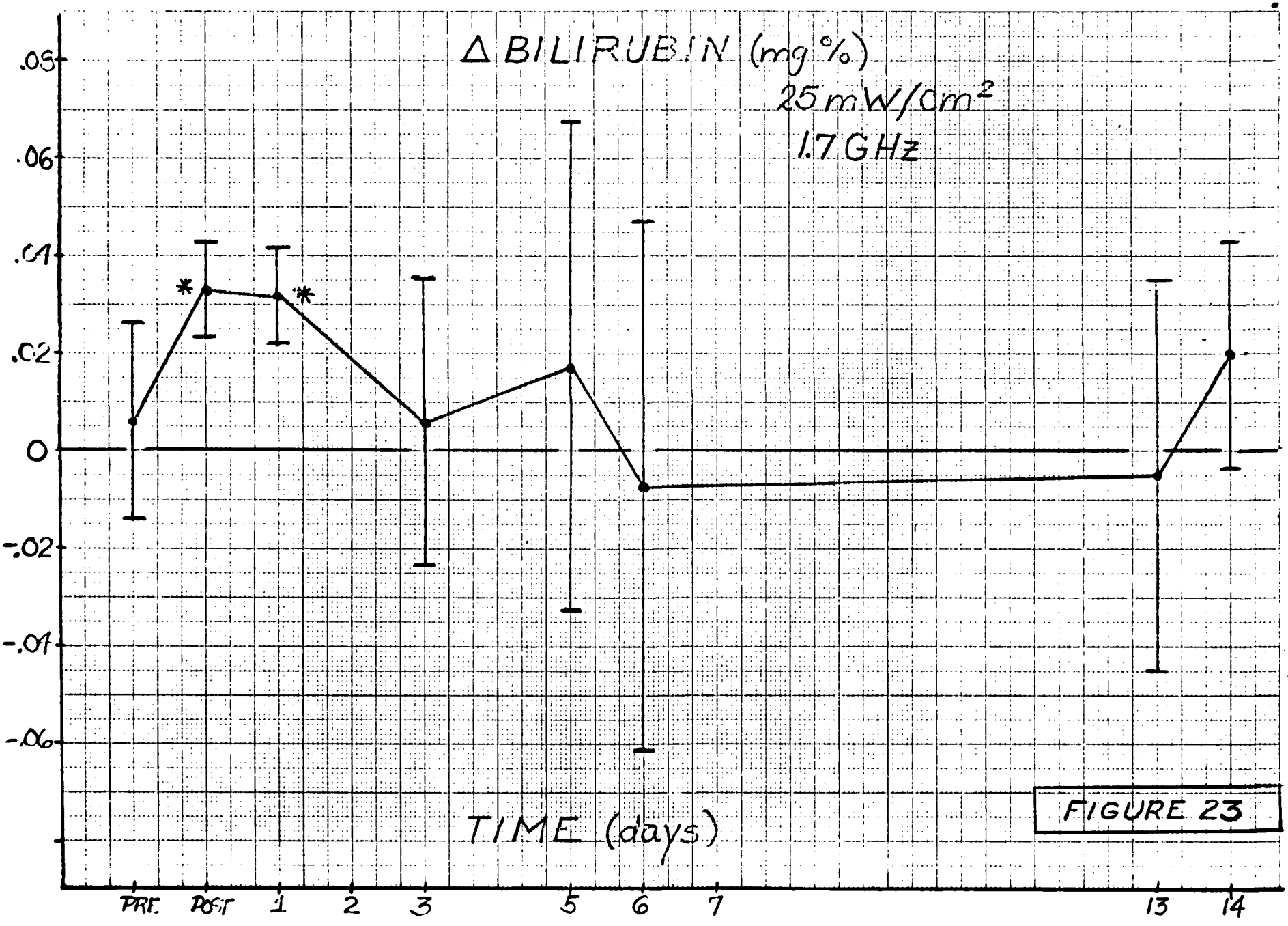
ΔBILIRUBIN (mg %)
25 mW/cm²
1.7 GHz
.08
.06
.04
.02
0
-.02
-.04
-.06
*
*
TIME (days)
PRE.
POST
1
2
3
5
6
7
13
14

FIGURE 23

PREPRINT

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EFFECT OF MICROWAVE RADIATION ON PENTOBARBITAL-INDUCED SLEEPING TIME

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Department of Biophysics
Medical College of Virginia
Virginia Commonwealth University
Richmond, Va. 23298

ABSTRACT

A dose-dependent statistically significant decrease in the duration of sodium pentobarbital-induced sleeping time was elicited by exposure of Dutch rabbits to 1.7 and 2.45 GHz microwave radiation in the intensity range of from 5 to 50mW/cm^2. The mechanism for sleeping time reduction has been investigated using rectal temperature and sleeping time as the dependent variables. Evidence is presented which indicates that the sleeping time alteration is related to the thermal stress imposed on the animal by microwave irradiation but alternative mechanisms cannot be ruled out at present.

INTRODUCTION

Although a large number of biological effects of microwave radiation have been reported in the literature, there are few cases in which the mechanisms of interaction are specified. Consequently, difficulty has been encountered in establishing microwave exposure limits for humans since the significant variables have not been determined. In the present study of the effects of microwave radiation on the duration of sodium pentobarbital sleeping time, an attempt has been made to determine the primary mechanism of interaction of the electromagnetic field with the biological subject, the Dutch rabbit, which leads to reproducible alterations in the response variable. Drug-induced sleeping time was selected as the dependent variable for this study since it is well known, simple, and easily replicated method of investigating the effects of physiological or metabolic stress in mammalian systems. The somewhat limited precision of this response variable is easily compensated for by proper experimental design.

The dependent variables investigated were the duration of sodium pentobarbital induced sleeping time and the rectal temperature; independent variables included microwave frequency (2.45 and 1.7GHz), microwave intensity, and ambient temperature. Additional studies, which will not be reported here, involved exposure to pulse modulated microwave fields and variations of drug dosage.

MATERIALS AND METHODS

Three weeks prior to microwave exposure 8 to 10 age-matched, litter-mate Dutch rabbits (1.6 to 2.8kg) were anesthetized with sodium pentobarbital at a dosage level of 22mg/kg. The anesthetic, a general nonselective central

nervous system depressant, was injected into the marginal ear vein of the rabbit during a 2 minute period, producing a rapid onset of anesthesia characterized by the complete loss of the righting reflex, the criteria for the start of the sleeping time. The duration of drug-induced sleep was defined as the time required for the animal to regain the righting reflex. Group mean sleeping times and standard deviations were determined under sham-irradiation conditions at normal room temperature (22°C).

The biological elimination rate of sodium pentobarbital is 0.55 day^{-1} (1), thus a two to three week delay between sham and microwave exposure was used to minimize residual drug effects. In the microwave experiments the animals were placed in the microwave field immediately after the loss of the righting reflex; sleeping time was determined by observing the animals with closed circuit television. Animals were placed on their sides with their bodies oriented perpendicular to the microwave beam axis which intersected the midline of the animal at its ventral aspect. The animals were placed on a styrofoam support during irradiation in the far field of a horn antenna in an anechoic chamber which was maintained at a temperature of 22 $\pm$ 0.6°C and a relative humidity of from 40 to 60%. All sham irradiated animals were subjected to the same conditions without the presence of a microwave field. The microwave electric field was vertically polarized in the experiments reported here. In order to detect the possible effect of long-term drug tolerance due to the repeated administration of pentobarbital, post-exposure sleeping times were also determined for the same group of animals 2 to 3 weeks after microwave exposure. The group mean sleeping times for pre and post-exposure sham irradiations were compared statistically to assure that drug tolerance was not affecting the microwave response of the animals.

Rectal temperatures were measured by the use of a thermistor (Yellow Springs Instrument Co. Model 702) with a digital thermometer readout (Digitec) The thermistor, which had a time constant of approximately 1 second, was inserted 5 cm into the rabbit colon during measurement of rectal temperature. Temperatures were measured immediately prior to anesthesia and again 15 to 30 seconds after the righting reflex was regained and the microwave power was turned off; total measurement time was 90-120 seconds. Rectal temperature cooling curves for microwave exposed rabbits were used to estimate the maximum error due to the time lag between cessation of microwave exposure and the temperature measurement. The maximum error was determined to be less than 0.1°C and in consideration of the magnitude of this error with respect to other experimental errors, the rectal temperature data has not been corrected.

The rectal temperature-time histories of microwave and sham irradiated anesthetized Dutch rabbits were also determined. Sham irradiation temperature records were obtained by continuous rectal temperature recordings during the course of the sleeping time. In the case of microwave irradiation of anesthetized animals, it was not feasible to continuously monitor the rectal temperature due to perturbations caused by the presence of the thermistor probe in the electromagnetic field. In this case, therefore, the microwave field was turned off for a period of 1 to 2 minutes during which time the temperature was measured. Using a number of different animals and measuring at 15 minute intervals, it was thus possible to estimate the mean rectal temperature increase in anesthetized Dutch rabbits as a function of irradiation time with an error of less than 1%. Using a semi-empirical theoretical method based upon a heat balance and the experimental temperature data, a relationship was established between the rectal temperature rise, irradiation time, and microwave power density. This relationship was subsequently used to relate alterations in sleeping time to microwave induced thermal stress in the Dutch rabbit.

The irradiations were conducted at a frequency of either 2.45GHz or 1.7GHz, in the far-field of a standard gain horn antenna in a temperature-controlled anechoic chamber at the Walter Reed Army Institute of Research. Free space power density calibrations were performed with matched standard gain horns and a dipole antenna was periodically used as a field intensity calibration check. Calibrations were provided by the staff of the Department of Microwave Research, Walter Reed Army Institute of Research. Continuous wave (CW) microwave irradiations were administered at a distance of 10.5 feet from the antenna aperture to the midline of the animal. The lateral field distribution at this distance was determined by the use of a dipole. At the animal's midline in air the power density variation was less than 10% over the entire length of the animal and the vertical field variation over the supine animal's height was less than 5%. The free space CW microwave power densities used in this study were 5, 10, 15, 25 and 50mW/cm^2 at either 2.45 or 1.7GHz.

The effect of elevations of the ambient temperature on sleeping time was investigated in another series of experiments in which Dutch rabbits were anesthetized and placed in a temperature controlled environmental chamber at 22^o $\pm$ 0.6^{o}C or 39^o $\pm$ 1^{o}C. Rectal temperatures were monitored continuously during these exposures as described above. The four types of exposure conditions used in this study were thus: 1) sham-microwave exposure at room temperature (22^{o}C) in an anechoic chamber, 2) microwave exposure at room temperature (22^{o}C) in an anechoic chamber, 3) exposure at room temperature (22^{o}C) in an environmental temperature-controlled chamber, and 4) exposure to an elevated ambient temperature of 39^{o}C in an environmental temperature-controlled chamber.

The statistical significance of the differences in group mean sleeping times and rectal temperature elevations under these exposure conditions was determined by means of the two-tailed Student's t test. Correlations between study variables were determined by regression analysis. The level of statistical significance used as a criterion for rejection of the null hypothesis was the 5% level (ie. $p < 0.05$).

RESULTS

Microwave exposure of anesthetized Dutch rabbits at either 1.7 or 2.45GHz resulted in a statistically significant dose dependent decrease in sleeping time. Exposure to 2.45GHz CW microwaves, as previously reported by Wangemann and Cleary (2), resulted in a significant analeptic effect at power densities of 5mW/cm^2 or greater. At a frequency of 1.7GHz no reduction in sleeping time was detected at 5mW/cm^2 but for intensities of 10mW/cm^2 or greater, statistically significant reductions in sleeping time of from 30 to 70% were obtained. The results of this series of determinations are summarized in Figure 1, a plot of mean sodium pentobarbital sleeping time as a function of microwave power density. All mean sleeping times shown in this figure were statistically significantly (ie. $p < 0.05$) reduced with respect to the pre or post-exposure room temperature sham irradiation means, with the exception of the 5mW/cm^2, 1.7GHz mean. The error bars in this figure represent $\pm$ one standard error of the mean and the lines connecting the means are drawn to facilitate comparison of the dose-responses for the two microwave frequencies employed in this study.

It may be noted that in the range of power densities used in this study, 2.45GHz irradiation resulted in a greater analeptic effect than 1.7GHz microwaves. The difference in the mean sleeping time at 2.45 GHz compared to 1.7GHz was highly statistically significant at 10 and 25mW/cm^2 and the lack of significance at the other power densities is attributed to the relatively smaller sample sizes at these intensities. Considering the comparison of the mean

sleeping times at 2.45 and 1.7GHz at each power density as mutually independent statistical tests, the probabilities associated with the differences at each power density may be combined according to the general method described by Fisher (3). Combination of probabilities in this manner reveals that the overall difference between the mean sleeping times at 2.45 and 1.7GHz is highly statistically significant ($p < .001$) suggesting that 2.45GHz microwave radiation produces a more pronounced analeptic effect than 1.7GHz radiation in the range of intensities of from 5 to 50mW/cm^2.

The reduction in sleeping time with increasing microwave power density suggested that thermal stress may alter the normal duration of anesthesia in the Dutch rabbit. A significant microwave intensity dependent increase in rectal temperature was detected for intensities of 10mW/cm^2 or greater for both 2.45 and 1.7GHz radiation. Application of a thermal balance to data obtained for the mean rectal temperature rise in a group of 4 Dutch rabbits as a function of time of exposure to 1.7GHz microwaves at an intensity of 25mW/cm^2 resulted in the semi-empirical relationship between rectal temperature change (ΔT), microwave power density P, and irradiation time t indicated by Equation 1.

$$\Delta T = 0.13P \{1 - \exp(-0.0132t)\} \quad (1)$$

This equation describes the rectal temperature rise during the period in which the thermal compensating mechanism of the animal is capable of maintaining thermal equilibrium. For the Dutch rabbit under these irradiation conditions this period is approximately 2 hrs. (2). A comparison of the rectal temperature rise predicted by Equation 1 with experimental data obtained at 1.7 GHz at an intensity of 10mW/cm^2 is shown in Figure 2. Although the experimental and theoretical curves are not coincident at this power density, the general agreement suggests that Equation 1 may be used to estimate the dependence of the rabbit rectal temperature elevation on microwave power density. Also shown in Figure 2 is the temporal behavior of the rectal temperature of an unanesthetized Dutch rabbit during sham-irradiation conditions. Although there is a detectable increase in rectal temperature during sham exposure, the magnitude of the change is significantly less than that resulting from microwave exposure at 10mW/cm^2 or higher intensities.

In another series of experiments the mean rectal temperature change in anesthetized rabbitswas determined as a function of 1.7GHz microwave power density. The rectal temperature change, in this case, was defined as the difference between the rectal temperature at the time the anesthetized animal regained the righting reflex (ie. at the termination of the "sleeping time") and the pre-anesthesia rectal temperature. The mean rectal temperature change, thus defined, is shown as a function of power density in Figure 3. At a power density of 5mW/cm^2 the mean ΔT for anesthetized animals is the same as unanesthetized sham-exposed rabbits. No reduction in sleeping time was detected at this intensity for 1.7GHz microwaves.

For intensities of 10mW/cm^2 or greater, highly statistically significant ($p < 0.001$) rectal temperature increases were induced as a consequence of exposure, as were significant reductions in pentobarbital-induced sleeping times as shown in Figure 1. A comparison of these results suggests that the underlying mechanism for the reduction in sleeping time is dependent upon microwave-induced thermal stress as reflected by alterations in the rectal temperature. To further investigate the interaction of sleeping time and thermal stress the relationship between sleeping time (t_s) and rectal temperature change (ΔT) in Dutch rabbits irradiated with 1.7GHz microwaves at intensities in the range of from 10 to 50mW/cm^2 was determined by linear regression analysis. Sleeping time was found to be related to rectal temperature change by the equation

$$t_s = 50 - 21.4 \ \Delta T, \qquad (2)$$

where t_s is the sleeping time in minutes and ΔT is the rectal temperature change in °C. By redefining the time variable (t) in Eq. 1 as the sleeping time (t_s) and eliminating ΔT from Equations 1 and 2, a relationship between microwave power density (P) and sleeping time may be derived as given by Equation 3.

$$P = \{17.9 - 0.36\ t_s\}\ \{1 - \exp(-0.0132 t_s)\}^{-1} \qquad (3)$$

Equation 3 is plotted in Figure 4 together with the experimental data relating pentobarbital sleeping time to the power density of 1.7GHz microwave radiation. Although not shown in this figure, similar results were obtained in the case of 2.45GHz radiation. The agreement of the experimental data with the results predicted by Eq. 3, as shown in Figure 3, suggests that the microwave power density dependent decrease in sleeping time is related to radiation-induced thermal stress as manifested by alterations in rectal temperature.

To further investigate the role of thermal stress in sleeping time alterations, the relationship of rectal temperature to sleeping time in unirradiated pentobarbital anesthetized rabbits maintained at normal room temperature of 22 ± 0.6°C or at an elevated ambient temperature of 39 ± 1°C was determined. The mean rectal temperatures for two groups of rabbits under these conditions are shown as a function of time in Figure 5. At 22°C a significant decrease in rectal temperature of approximately 0.5°C was detected in contrast to the results obtained at 39°C which indicated a time dependent increase in rectal temperature to a mean level of approximately 1°C after one hour of exposure at that temperature.

The depression of the rectal temperatures of anesthetized animals kept at room temperature is an anticipated response to a CNS depressant such as sodium pentobarbital. The increase in the rectal temperature of anesthetized rabbits exposed to an ambient temperature of 39°C immediately after anesthetization is consistent with the elevation induced by microwave exposure at 10mW/cm^2. A comparison of the mean sleeping times for the 22°C and 39°C groups shown in Fig. 5, however, indicates that exposure to an elevated ambient temperature, which led to a 1°C rectal temperature increase did not result in a decrease in sleeping time in contrast to the results of microwave exposure. In a subsequent replication of this experiment, there was a decrease in the mean sleeping time for rabbits exposed to 39°C, relative to animals maintained at 22°C, but the difference in the means was not statistically significant ($p > 0.05$). Even though the degree of thermal stress, as reflected in the mean rectal temperature increase at the end of the sleep period was approximately the same for animals exposed to 1.7GHz microwaves at an intensity of 10mW/cm^2 or to an ambient temperature of 39°C, microwave irradiation produced a significantly greater reduction in sleeping time. It must be noted that the nature of the thermal stress in these cases differed as a result of variations in the distribution of absorbed energy within the animals and in the rate of energy absorption. One measurable difference of the effect of microwave versus ambient temperature induced thermal stress was the rate of rectal temperature increase. In anesthetized Dutch rabbits exposed to 1.7GHz microwaves at 10mW/cm^2 the mean rate of rectal temperature increase was 0.034 ± 0.008 °C/min. as compared to a mean rate of increase of 0.018 ± 0.003 °C/min for anesthetized animals at an ambient temperature of 39°C

DISCUSSION

The results of this investigation indicate that the duration of pentobarbital-induced sleeping time is decreased as a consequence of exposure to 1.7 or 2.45GHz microwave radiation. The magnitude of the sleeping time reduction is related to the field intensity for fields that produce detectable increases in rectal temperature, suggesting that the effect involves thermal stress due to microwave absorption. The mechanisms whereby thermal stress affects sleeping time are dependent upon several factors related to the mode of action of anesthetic agents in the CNS and the distribution and metabolism of such drugs.

The state of anesthesia occurs when a critical concentration or a critical molar volume of an anesthetic agent is achieved in the neuronal membranes. General anesthetics, such as pentobarbital, depress postsynaptic excitatory transmission in the vertebrate central and peripheral nervous system, while preserving or prolonging presynaptic and postsynaptic inhibition. Ransom and Barber (4) have demonstrated that pentobarbital produces three specific effects on mammalian CNS neurons. Postsynaptic glutamate excitation is depressed independently of direct effects on membrane potential or conductance. The drug also induces a variable prolongation of the conductance change induced by the neurotransmitter γ-amino-butyric acid and it also produces a slow dose-dependent increase in membrane conductance. The authors conclude that the depression of excitatory postsynaptic potentials in the CNS by pentobarbital could thus be the result of the selective postsynaptic effects of the drug without necessitating major effects on presynaptic release. It is also indicated that pentobarbital does not directly alter neuronal membranes to any extent (4). On the basis of these findings, microwave induced alterations in pentobarbital action manifested in a decrease in sleeping time could involve either a direct effect upon postsynaptic neuronal processes or changes in the CNS drug concentration due to alterations in drug distribution or metabolism.

Pharmacological studies of the effect of environmental temperature on sleeping times have revealed that a rise in temperature tends to shorten sleeping times for barbiturates such as pentobarbital which are metabolized by the organism as contrasted to those which are eliminated unchanged or metabolized to a slight extent which show either no temperature dependence or an increase in sleeping time at increased temperature (5). The effect of increased temperature has been attributed either to an increase in the rate of drug catabolism (6) or to alteration in the mode of distribution (7).

Setnikar and Temelcou(5)have shown that at a lowerenvironmental temperature (15°C) the diffusion of pentobarbital from blood to tissue and the metabolism were decelerated and the volume of distribution was decreased relative to temperatures of 30°C in rats and dogs. Exposure to the cold environment reduced the liver-blood and brain-blood repartition coefficients of pentobarbital, but the changes were not directly correlated with body temperature. In rats it was determined that at the 5th minute after administration,the concentration of pentobarbital was 1.16 times higher in rats at 30°C than at 15°C. By the 15th and 30th minute the opposite was found with ratios of 0.76 and 0.67 respectively (5). Since the anaesthetic effect depends upon the concentration in the brain, sleeping times become shorter in animals kept at high room temperature due to the greater rate of elimination as a result of increased metabolism and an increased rate of diffusion from brain tissue. The effect of temperature on the metabolism of pentobarbital has also been investigated by Kalser and co-workers (8). They found that metabolites formed by the liver are excreted into blood rather than bile, a finding in accord with observations indicating excretion of pentobarbital metabolites in the urine. Hypothermia markedly decreased the amount of metabolites appearing in both blood and bile

as well as liver which is compatible with a decreased rate of metabolism in that organ.

Goldstein and Sisko (9) studied the effects of 9.3GHz microwaves at intensities in the range of from 0.7 to 2.8 mW/cm^2 on the electroencephalographic patterns of rabbits anesthetized with pentobarbital at a dosage of 4mg/kg. They found that following a latent period of 3 to 12 min after exposure there was a sudden arousal lasting for an average of 3 min. This was followed by a return to sedation for 3 to 5 min after which a second period of arousal 2 to 10 min in duration occurred, followed in some cases by up to 2 additional periods of arousal. The duration of arousal, which was significantly increased by microwave irradiation, indicated a possible dose-related response. Thermal effects were ruled out due to the magnitude of the microwave field and the latent period following exposure before the effect was detected. Goldstein and Sisko (9) comment on the similarity between the microwave response and effects observed after acute injections of naturally occurring compounds chemically modified to contain free radicals. They suggest, on this basis, that microwave radiation may produce or enhance the formation of free radicals from naturally occurring compounds in the brain.

Pharmacological effects of 3GHz pulsed microwave radiation at an average power density of $5mW/cm^2$ were reported by Servantie and co-workers (10). Although these authors did not report on pentobarbital effects, they suggest a mechanism for microwave-drug interactions. Studying the effect of prolonged irradiation on the susceptibility of rats to pentetrazol administration, they found that after 15 days of microwave exposure the onset of drug-induced convulsions was supressed in irradiated animals. For longer exposures, particularly 27 days or more, the susceptibility to convulsions as well as mortality increased in the irradiated animals. The effect of microwave exposure on the action of curare-like drugs was also investigated by these authors (10). Using intact animals, in situ preparations, and isolated preparations, it was determined that microwave exposure decreased the susceptibility of rats to the effects of paralyzing drugs. Of the possible mechanisms considered by the authors it was concluded that the most probable explanation was that the microwave field acted upon the neuromuscular synapse at the post synaptic membrane to cause a decrease in the binding energy between the drug molecule and the enzyme acetylcholinesterase.

Studies of the effects of microwave exposure on <u>in vivo</u> pharmacological alterations thus suggest the possibility of direct mechanisms of interaction between the field and either the drug molecule or other molecules present in the CNS or peripheral nervous system (9,10). The microwave intensities employed in these studies, as well as the nature of the alterations, suggested to the authors that the effects were not directly attributable to microwave-induced heating. Alterations in the arousal response of rabbits, as described by Goldstein and Sisko (9), are in qualitative agreement with the results of this study since the duration of sleeping time is related to the arousal status of the animal. The findings of Servantie et.al. (10), that microwave exposure decreases the susceptibility of animals to the effects of muscle paralyzing agents, are also consistent with the decreased effectiveness of pentobarbital anesthesia detected in this study, thus suggesting that the reduction in sleeping time resulting from 1.7 and 2.45GHz microwaves could involve direct microwave effects on nervous tissue.

However, considering the microwave intensity dependence of the reduction in sleeping time and the fact that in all instances, except one, there was a detectable elevation in rectal temperature associated with sleeping time reductions, it is not possible to conclude that the reductions were not related

to microwave-induced thermal stress. Irradiations at 2.45GHz at an intensity of 5mW/cm^2, which led to a statistically significant decrease in sleeping time, did not involve a detectable increase in the mean rectal temperature, but this does not rule out the possibility of a certain low level of thermal stress. It should also be noted that Goldstein and Sisko (9) detected microwave-induced arousal only in cases in which the relative humidity was less than 40%. In the present study, all experiments were conducted at a relative humidity of 40% or greater.

The dependency of pentobarbital sleeping time, drug-tissue distribution and metabolism, on environmental temperature as described in references (5) to (8) are in agreement with a mechanism of a thermally induced decrease in the concentration of pentobarbital in the rabbit brain and increased drug catabolism in the liver. Pending the results of future investigations of the effects of microwave exposure on pentobarbital distribution and metabolism, it is tentatively concluded that the major factor leading to the reduction in sleeping time resulting from microwave exposure is thermal stress. The fact that sleeping time was reduced at 5mW/cm^2 for a 2.45GHz field and the significant difference between the effects of 2.45 and 1.7GHz irradiations suggests that the absorbed energy distribution within the experimental animal significantly affects drug distribution and absorption. This is further suggested by the fact that exposure at 39°C did not result in as significant a reduction in sleeping time as microwave exposure at a power density that resulted in the same rectal temperature elevation.

CONCLUSIONS

Microwave irradiation at frequencies of 2.45 and 1.7GHz results in statistically significant reductions in sodium pentobarbital sleeping time at intensities of from 5 to 50mW/cm^2. Increases in rectal temperatures of irradiated animals were correlated with the sleeping time reductions and a semi-empirical thermal response model has been developed to describe the relationship between microwave power density and sleeping time. It is thus concluded that the results obtained in this study may be interpreted as a thermal stress response. The extent to which direct microwave interactions in the CNS, at the molecular or membrane level, contribute to the reduction in sleeping time is unknown and will be the subject of future research.

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FIGURE TITLES

Figure 1. Mean and standard error of the mean pentobarbital sleeping time (22mg/kg) as a function of 1.7 and 2.45GHz CW microwave free space power density.

Figure 2. Mean and standard error of the mean rabbit rectal temperature elevation versus irradiation time; 1.7GHz CW microwaves, 10mW/cm^2 free space power density.

Figure 3. Mean and standard error of the mean rabbit rectal temperature rise as a function of 1.7GHz CW microwave power density. Arrows indicate the range in the rectal temperatures at power densities for which only 2 data points were available.

Figure 4. Pentobarbital sleeping time in the Dutch rabbit as a function of 1.7GHz CW microwave power density. Comparison of experimental data (mean and standard error) and a semi-empirical relationship derived from a heat balance.

Figure 5. Mean and standard error of the mean rabbit rectal temperature change as a function of time post anesthesia for animals in a 39°C environmental chamber or in a 23°C environmental chamber.

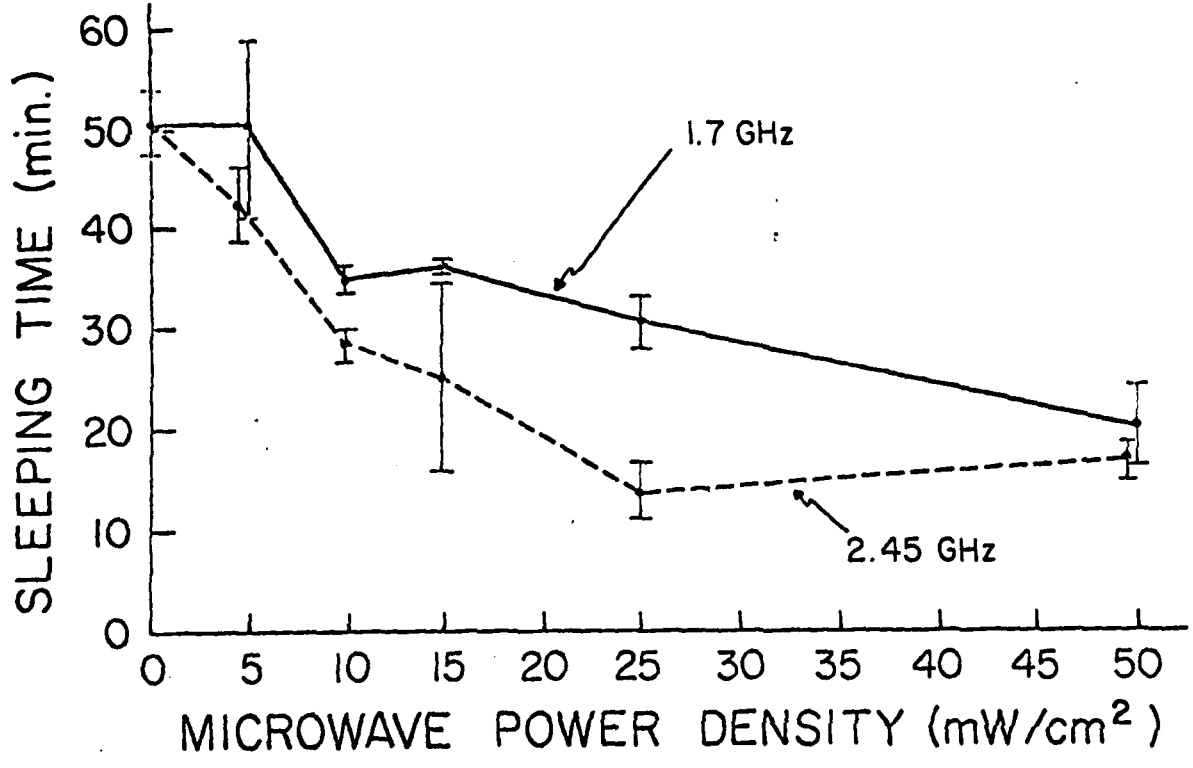

SLEEPING TIME (min.)
60
50
40
30
20
10
0
1.7 GHz
2.45 GHz
0
5
10
15
20
25
30
35
40
45
50
MICROWAVE POWER DENSITY (mW/cm²)

Fig. 1

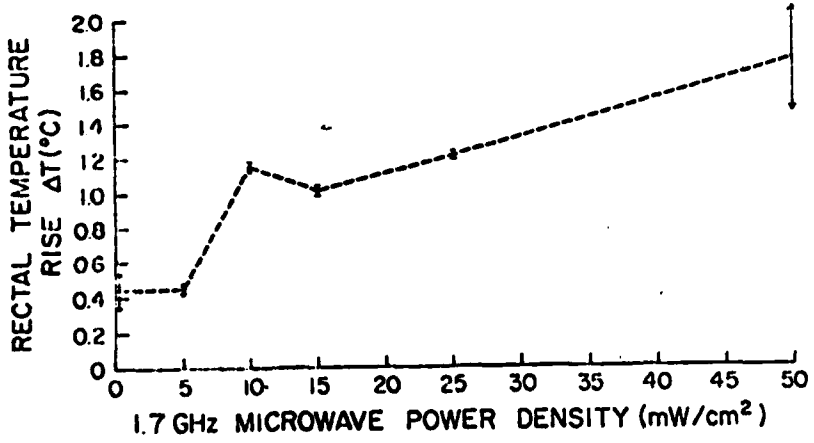

RECTAL TEMPERATURE RISE ΔT(°C)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0 5 10 15 20 25 30 35 40 45 50
1.7 GHz MICROWAVE POWER DENSITY (mW/cm²)

Fig. 3

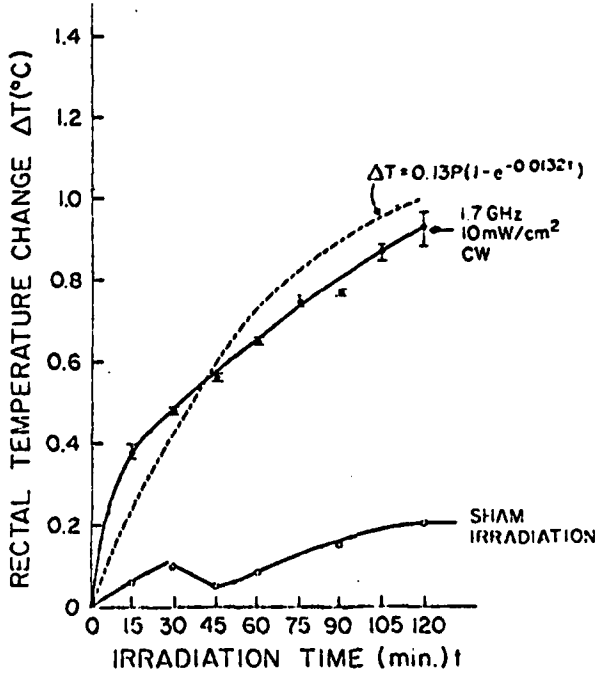

RECTAL TEMPERATURE CHANGE ΔT(°C)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
ΔT = 0.13P(1 - e^-0.0132t)
1.7 GHz
10mW/cm²
CW
SHAM
IRRADIATION
0 15 30 45 60 75 90 105 120
IRRADIATION TIME (min.) t

Fig. 2

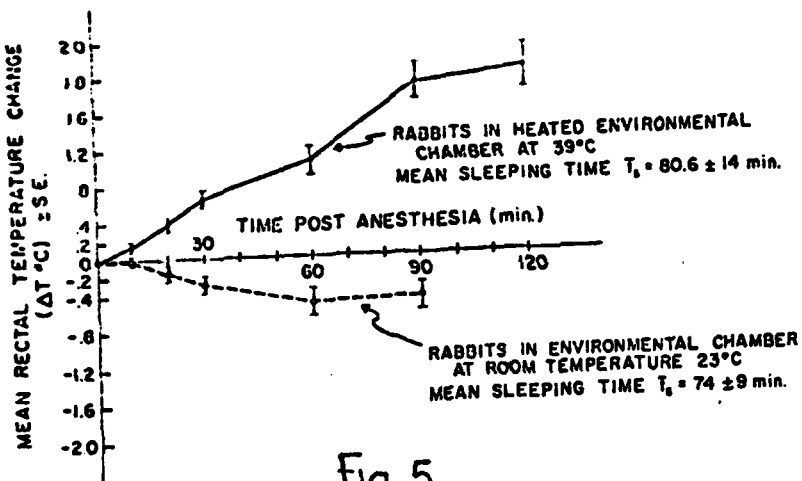

MEAN RECTAL TEMPERATURE CHANGE (ΔT °C) ±S.E.
2.0
1.6
1.2
0.8
0.4
0
-0.4
-0.8
-1.2
-1.6
-2.0
30
60
90
120
TIME POST ANESTHESIA (min.)
RABBITS IN HEATED ENVIRONMENTAL CHAMBER AT 39°C
MEAN SLEEPING TIME $\bar{T}_s$ = 80.6 ± 14 min.
RABBITS IN ENVIRONMENTAL CHAMBER AT ROOM TEMPERATURE 23°C
MEAN SLEEPING TIME $\bar{T}_s$ = 74 ±9 min.

Fig.5

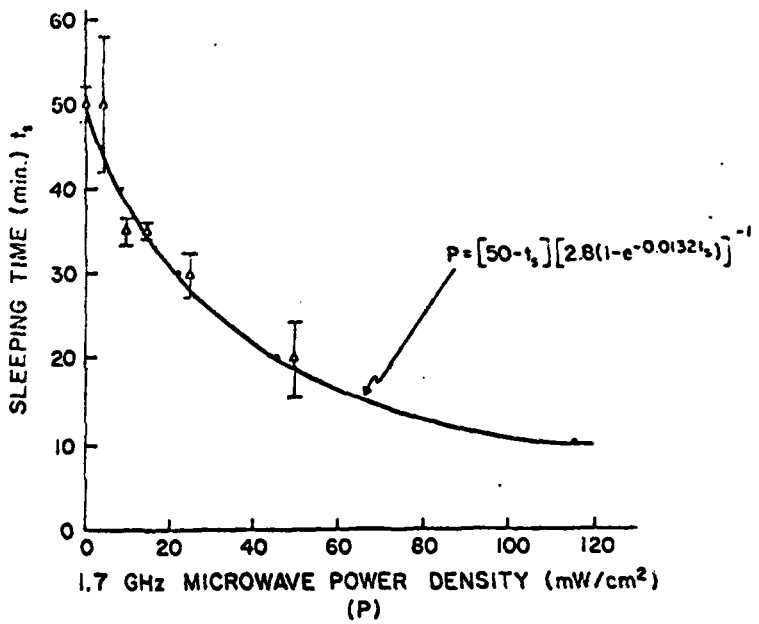
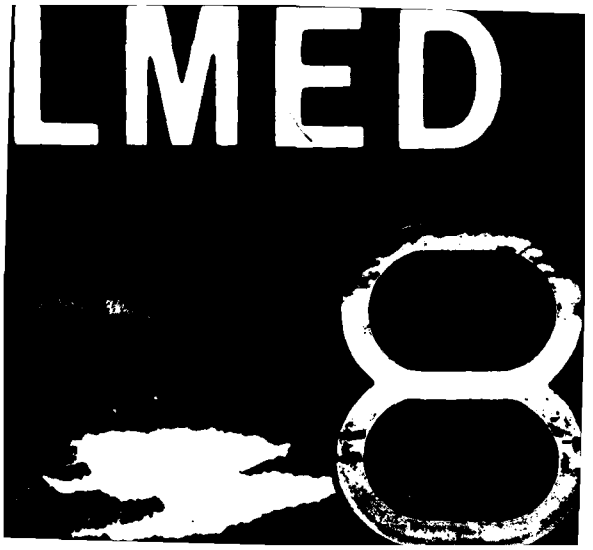

SLEEPING TIME (min.) t_s
60
50
40
30
20
10
0
0
20
40
60
80
100
120
$P=[50-t_s][2.8(1-e^{-0.0132t_s})]^{-1}$
1.7 GHz MICROWAVE POWER DENSITY (mW/cm²)
(P)

Fig.4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